The Evolutionary Potential of Male Plumage Color in a Hybrid Sparrow Species

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Preface

This thesis was written at the Centre for Ecological and Evolutionary Synthesis (CEES), at the Department of Biology, University of Oslo, under the supervision of Glenn-Peter Sætre, Anna Runemark and Richard Ian Bailey.

I truly appreciate the collective efforts of my team of supervisors; I honestly don't think I could have asked for a better team. I would like to thank Glenn-Peter for giving me this great opportunity, as these last two years has been a life experience that I shall never forget. Glenn's passion for evolutionary biology, as well as his exceptional ability to think outside the box, has been an inspiration. I would also like to thank Richard for showing me the true wonders of biostatistics. Richard's knowledge of statistics and R knows no bounds; I have learned so much from working with him, and his teaching has also led me to develop a passion for statistics and a drive to learn more. I want to thank Anna for her endless patience and her drive for excellence; she has motivated me to work hard and achieve goals. She is terrific at analyzing and conveying information biologically, in ways that are easy to understand, which has taught me so much related to biology and scientific writing. I feel lucky to have had Anna, Richard and Glenn as my advisors.

I would like to thank the whole sparrowgroup for being a pleasure to work with; they show great interest whenever anyone present results, and participate with thoughtful input. I also appreciate all my friends at the biology department whom I could discuss all aspects of biology with. Further, I send my warmest thanks to my friends and family for supporting me through this project.

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Abstract

Hybridization is increasingly recognized as a source of novel variation, but how hybridization can contribute to evolution is still not fully understood. Insights into the evolutionary potential of hybrid species, and the extent to which two parent species are constrained to form only a single specific hybrid phenotype, would increase our understanding of the impact of hybridization on evolution, adaptation and diversity. Here, I address these questions using the Italian sparrow (*Passer italiae*), a homoploid hybrid bird species formed from hybridization between house (*P. domesticus*) and Spanish sparrows (*P. hispaniolensis*). Using multivariate quantitative genetics methods, I examine four sexually dimorphic plumage traits (crown, back, rump and cheek) in males of all three species. For Italian sparrows, I compare three geographically separated island populations, which differ in genomic composition, to examine whether these populations vary in phenotype and whether they have more potential to evolve and diversify than the parent species. I show that the Italian sparrow is mosaic in its plumage pattern; some traits are similar to one of the parent species, others are similar to the other parent, but the Italian sparrow also has transgressive color values for back and rump (that is, outside the phenotypic range of either parent). In spite of strong parallelism in some traits, such as crown, island populations differ in some plumage traits, revealing that more than one phenotype can be formed by the same parent species. Alongside phenotypic novelty, the Italian sparrow has higher variability and evolvability than the parent species, showing that hybridization can indeed act as a source of new variation. However, my results indicate that, after initial hybridization, selection on secondary sexual signals can be strong along the axis of parental divergence in a hybrid species, as suggested by a pattern of low evolvability along this axis and fixation for one of the parental values for some traits in the Italian sparrow. This selection may be determined by inheritance patterns of parental female preferences in the hybrid species.

Introduction

According to the theory of evolution, the gradual buildup of heritable differences between populations eventually leads them to evolve into separate species with a common ancestor (Darwin, 1859). Due to natural and sexual selection, only a proportion of a population survives and reproduces, such that the frequency of alleles changes in the population over time (Futuyma, 2009). Genetic variation is the material upon which this selection acts, and the generation of new genetic variation is thus a crucial factor affecting the potential to respond to selective pressures.

New genetic variation can arise through mutation (e.g., Hoekstra et al., 2006). However, mutations are rare for each genetic locus and are often neutral (Kimura, 1985) or deleterious (e.g., Sawyer et al., 2007). Another way to create new variation is through hybridization. Even if pre- and postzygotic barriers impede two species from interbreeding and producing fertile offspring, total reproductive isolation takes a very long time to develop. Species that are not completely reproductively isolated may produce hybrids, and hybridization has been shown to occur in 10% of all animal and 25% of all plant species (Mallet, 2005). Hybrids are often inviable, sterile or have reduced reproductive success (e.g., Ålund et al., 2013). However, even if hybridization is infrequent and hybrids have reduced reproductive success, favorable alleles can be transferred between species via introgression from rare hybridization events (e.g., Song et al., 2011, Pardo-Diaz et al., 2012). Unlike mutation, genes transferred by hybridization have already been tested by selection and are therefore less likely to be deleterious (Rieseberg et al., 2003). Moreover, while mutations are often small-scale, hybridization can transfer entire genes or even gene complexes into a species. Indeed, beyond transfer of genetic material between species, whole hybrid populations can arise from a complete mixing of parental genomes. Therefore, the effect on evolutionary potential from hybridization is expected to differ from that for mutations.

Hybridization also has the potential to produce new species. In hybrid speciation, the interbreeding of distinct but not fully reproductively isolated species results in the evolution of a third, hybrid species (Mallet 2007). There are two forms of hybrid speciation, polyploid hybrid speciation, where chromosome number is increased in the hybrid, and homoploid hybrid speciation (HHS) where there is no change in chromosome number.

Throughout the history of biological science, botanists have regarded hybrid speciation as an important evolutionary process, while zoologists have considered hybridization to be of little importance for evolution (Dowling and Secor, 1997). The ongoing genetic and genomic revolution has, however, shown that hybridization and introgression are more prevalent than previously thought (Mallet, 2005, Mallet, 2007, Abbott et al., 2013). Despite this, how hybridization can contribute to evolutionary potential is still not fully understood.

The influence of hybridization on diversity and evolutionary constraints

Two key ways in which hybridization can affect evolutionary potential are through altering genomic diversity, and through altering constraints on the direction of evolution. With respect to diversity, hybridization can introduce more additive genetic variation to a trait relative to the amount present in either of the parental species' genomes, and this increase in genetic variation is maximized when the parent species are fixed for different alleles at each locus. Hybrid populations inherit a unique, mosaic genome from the combination of the two parental genome complexes (Rieseberg et al., 2003), which may transcribe to novel phenotypes in several different ways. They can display phenotypes that are intermediate or mosaic versions of the parents (that is, some traits are similar to one of the parents, other traits are similar to the other), or extreme, so-called transgressive phenotypes, which are beyond the range of the parental species (Rieseberg et al., 1999, Dittrich-Reed and Fitzpatrick, 2013). Additive effects can cause transgression by increasing the trait variance in hybrid populations via complementary gene action (e.g., Vega and Frey, 1980). Complementary gene action does not affect the trait mean, which would therefore remain intermediate. Other factors might cause transgressive trait means, such as overdominance (e.g., Rick and Smith, 1953), heterosis (e.g., Vilà and D'antonio, 1998), epistasis (Rieseberg et al., 1999), or selection favoring extreme phenotypes in highly variable hybrid populations. The new, rearranged combinations of parental genes may increase the potential for hybrids to adapt and diverge (Eroukhmanoff et al., 2013), to the extent that novel phenotypes and mosaic phenotypic combinations can allow a hybrid species or population to occupy a novel ecological niche unsuitable for both parent species (e.g., Gompert et al., 2006). It is also possible for a hybrid to outcompete one or both of its parents in their own ecological niches (e.g., Ayres et al., 1999, Ellstrand and Schierenbeck, 2000).

Conversely, diversity in hybrids is often likely to be reduced by selection acting on genetic incompatibilities between the parents, leaving only a limited number of fit hybrid genotypes. Hybrids are often inviable or infertile (Dobzhansky, 1936, Orr, 1995, e.g., Neff, 2004). Incompatibilities can arise from chromosome number effects; failure of a division reduction during the recombination event may lead to a polyploid hybrid, a hybrid with three or more sets of chromosomes (Ramsey and Schemske, 1998). In animals, polyploidy is often fatal for the fetus (Muller, 1925), and odd-numbered levels of polyploidy severely reduce fertility in plants (e.g., Grant, 1953). Furthermore, alleles at different loci that are fixed in different directions in the parents might have conflicting functions and lower fitness when coupled in the hybrid (e.g., Dobzhansky, 1936). Such incompatibilities can cause inviability, reduced fertility or dysfunctional phenotypes in the hybrid (e.g., Burton, 1990, True et al., 1996). Extrinsic selection against hybrids can also arise and hence reduce hybrid genetic diversity, as hybrids may be less well adapted to both parental niches than respective parent species, and simultaneously not be adapted to a niche outside the parental range (Mallet, 2007).

New genetic variation is not always enough to induce evolution; it must also be available to selection (Bailey et al., 2013). The potential for a population to evolve may be constrained by its number of fixed trait combinations (Kirkpatrick, 2009). Individually, the traits may be fully capable of responding to selection, but together, the correlations of traits may confine a population to only evolve in specific directions (Hansen and Houle, 2008, Kirkpatrick, 2009, Walsh and Blows, 2009). Hybridization has the potential to alter trait genetic correlations by altering the levels of pleiotropy or linkage disequilibria among them (Bailey et al., 2013).

The strength and number of traits under selection will influence a population's ability to respond to selection. If a set of traits from a particular combination is selected for, while another set of traits from the same combination are selected against, evolution will stagnate for all the traits involved (Cheverud, 1996). This may be particularly relevant to hybrid evolutionary potential, due to the presence of incompatibilities and otherwise unfit genotypes and phenotypes, which may interfere with other forms of selection (Bailey et al., 2013). The possible genomic composition of a hybrid may have numerous incompatible combinations and few viable trajectories (Barton, 2001, Eroukhmanoff et al., 2013). Hybrids may be restricted to evolve only in the directions similar to their parental lineages, or towards fit ancestral gene combinations that existed prior to speciation (Gavrilets, 1997). They may also be constrained to inherit several specific sets of coupled trait combinations from the parent

species (observed in Hawthorne and Via, 2001) which, in turn, constrains their ability to evolve. In addition, there could be several different loci controlling the same trait, and they can act in different ways, requiring different triggers or having an unequal amount of dominance or additive effects, which may influence the ability to respond to selection (Blows et al., 2004).

How, and to what extent, the parental species have diverged may also influence the hybrid pattern of evolution (Stelkens and Seehausen, 2009, Eroukhmanoff et al., 2013, Bailey et al., 2013). If the parents are under divergent directional selection, hybrids are predicted to have an intermediate phenotype, and to be constrained to evolve along the parental axis of differentiation, with less potential to evolve novel phenotypes (Rieseberg et al., 1999, Bailey et al., 2013). Parental species under stabilizing selection are expected to produce hybrids with greater potential to evolve in a variety of directions, and have trait values extending beyond the parent taxa (Rieseberg et al., 1999, Bailey et al., 2013). Furthermore, there is evidence that hybrid phenotypes become more transgressive as the parents become more divergent (Stelkens and Seehausen, 2009). It is unclear, however, to what extent two parental genomes are constrained to only create one or a few variants of fertile hybrid or if different hybridization events between the same two parent species can result in different hybrid genotypes and phenotypes.

To address whether different phenotypes can arise from hybridization between the same two parent species I use the Italian sparrow (*Passer italiae*), which is a homoploid hybrid species that resulted from hybridization between the house sparrow (*P. domesticus*) and the Spanish sparrow (*P. hispaniolensis*) (Hermansen et al. 2011), as a model system. Three island populations of Italian sparrows from Corsica, Crete and Sicily (Fig. 1) differ in the proportion of the genome inherited from each parent species, which could suggest that each population arose from independent hybridization events (Runemark et al. *in preperation*). These independent island hybrid populations represent an excellent system to address whether a hybrid species is constrained to form a specific phenotype. The Italian sparrow has a plumage color pattern which is a mosaic of those of its parent species (Fig. 1) with an admixed genome shown to be mostly intermediate between the parents (Hermansen et al., 2011, Elgvin et al., 2011).

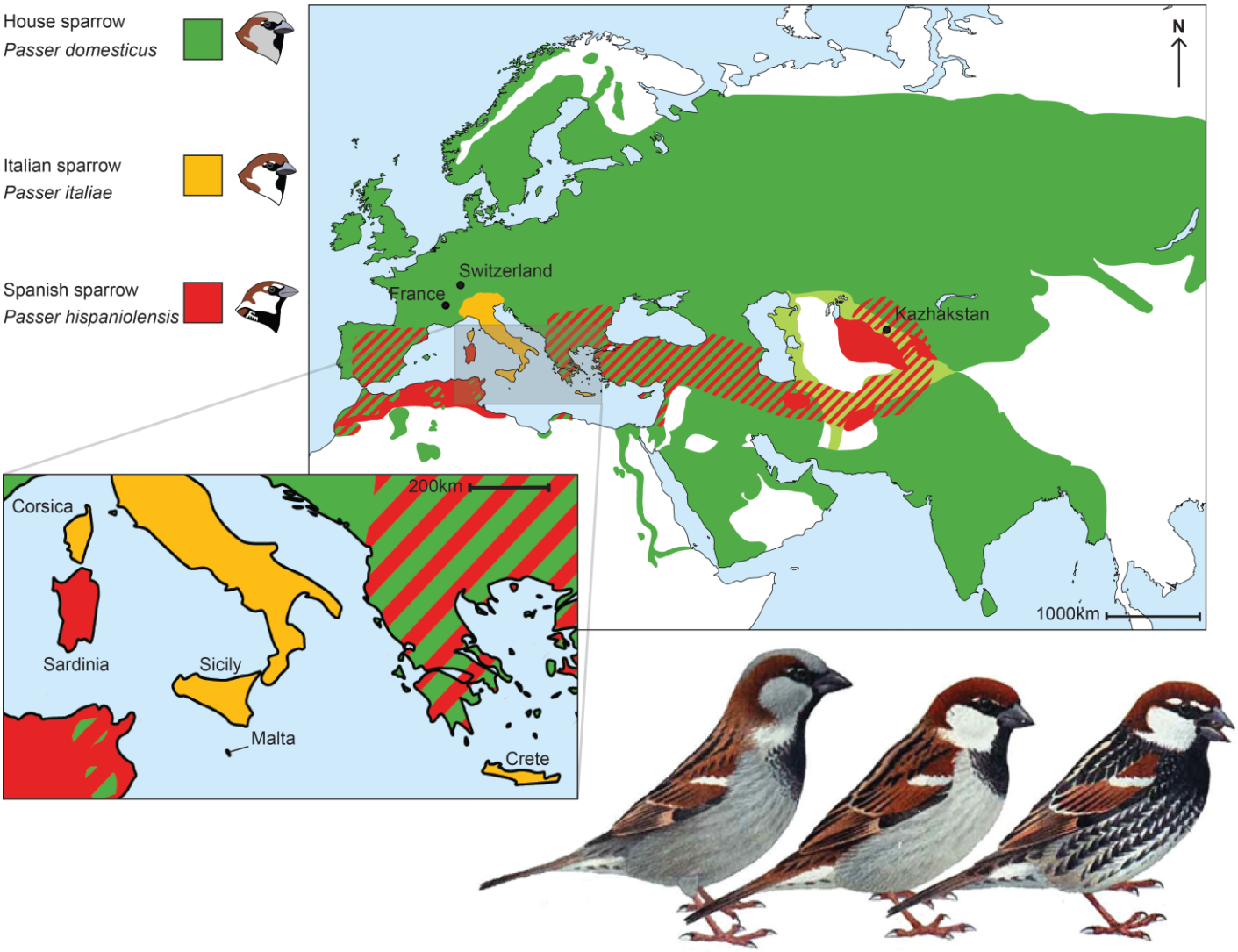


Figure 1. The distribution of house sparrows (non-commensal subspecies *P. d. bactrianus* in light green), Italian sparrows and Spanish sparrows across Eurasia, including a zoom-in map of islands Corsica, Crete, Malta, Sardinia and Sicily, and an image representing plumage color patterns of the sparrow system. From left to right; the house sparrow (*P. domesticus*), the Italian sparrow (*P. italiae*), and the Spanish sparrow (*P. hispaniolensis*). The Italian sparrow has a mosaic plumage color pattern of the two parental species. The head resembles the Spanish sparrow with a chestnut crown and white cheeks, while the rest of the body is more similar to house sparrows with a reduced bib, no flankings along the belly, brown and black back and grey rump.

For a hybrid species to establish, it has to develop reproductive isolation against its parent species (Mallet, 2007). This is particularly difficult to achieve for a homoploid hybrid species, since reproductive isolation needs to be developed between sympatric populations of parents and hybrids without the aid of an instantaneous postzygotic barrier that polyploidy provides (Trier et al., 2014, Hermansen et al., 2014). For successful HHS, the hybrid must either outcompete its parent species, or acquire a niche that is inaccessible to them, while simultaneously developing reproductive isolation. Nonetheless, several examples of HHS

exist in nature, and more are revealed as genetic data continues to be studied and new molecular genetic techniques continue to be developed (reviewed in Schumer et al., 2014, for examples see Rieseberg, 1991, James and Abbott, 2005, Schwarz et al., 2005, Gompert et al., 2006, Hermansen et al., 2011). As reproductive isolation against parent species is important for the establishment of homoploid hybrid species, it is of particular interest to study whether sexually selected traits are constrained to the same combinations in hybrid species.

Plumage color is often subject to sexual selection in birds (e.g., Andersson and Andersson, 1998, Pryke and Griffith, 2006) and plumage could hence be constrained by reinforcing sexual selection for certain phenotypic combinations to establish pre-mating isolation between the hybrid and parent species (e.g., Bailey et al., 2015). Assortative mating based on phenotypic traits can have aided in the establishment of reproductive isolation, and in turn, speciation (e.g., Mendelson, 2003). The Italian sparrow has a plumage color pattern that is strikingly intermediate between the two parent taxa (Summers-Smith, 1988, Töpfer, 2006). The male has a white cheek and chestnut crown like the Spanish sparrow, but no black flankings along the belly (Fig. 1). Compared to the house sparrow, the Italian sparrow lacks the gray crown but has the same brown-streaked back, reduced bib and grey rump. Females from all three species differ from the males in plumage color patterns, but have little discernable differentiation between species. This sexual dimorphism indicates sexual selection on male plumage, and studies have already indicated that sexual selection on male crown color – but not cheek color or eyebrow color – isolates the Italian sparrow from house sparrows across a narrow hybrid zone in the Alps (Bailey et al., 2015).

To differing extents, each plumage trait is made up of varying combinations of colors, and in some cases a mixture of colors produced by two different melanin types: eumelanin and pheomelanin (Prota et al., 1995). Hence, each trait individually has the potential to be both intermediate and mosaic with respect to parental plumage, and to vary in diversity and constraints between hybrid and parents. Isolated populations of Italian sparrows can thus differ in the amount of variability and evolve in different directions relative to each other and to the parent species. However, whether the Italian sparrow can achieve different plumage combinations or is constrained to form a certain phenotypic combination for instance due to selection, to be reproductively isolated from its parent species, is not known.

Here, I investigate evolutionary potential in male secondary sexual plumage traits in populations of the Italian sparrow from Corsica, Crete, and Sicily, and compare with their

parent species, house and Spanish sparrows, to infer the effects of diversity and constraints on evolutionary potential in a homoploid hybrid species. I use a standardized photography technique (Tesaker, 2014) and a novel approach for digital color quantification (Brydegaard et al., 2012) to obtain quantitative data on coloration as a basis for this analysis.

First, I investigate what the potential is for hybridization to form novel phenotypes. I study the degree of transgression, mosaicism and intermediacy in the plumage pattern of the Italian sparrow, and address if this differs between species and island populations. Second, I investigate what the relative effects of the changing diversity versus constraints are in a hybrid species. In particular, I analyze to what extent correlations within and between plumage traits (crown, cheek, back and rump) affect the evolutionary potential of the Italian sparrow differently from the parental species, and whether this varies by island. I also infer how much the plumage traits diverge between populations and islands relative to the amount of divergence in the parental species, as this may indicate the changing potential for future sparrow diversification with hybridization.

Materials and Methods

Sampling

Members of the research team, Anna Runemark, Jo Hermansen Fabrice Eroukhmanoff and Maria R. Tesaker, led by Glenn-Peter Sætre, sampled Italian sparrows on Corsica, Crete and Sicily in March-June 2013 (Fig.1). Three populations were sampled on each island; Cos, Enna and Naxos in Sicily, Chania Hospital, Istro and Mithinma Camping in Crete, and Muratello, Pianiccia and Tiuccia in Corsica. They caught altogether 171 male Italian sparrows from the three islands. Further, the research team caught Spanish sparrows in Sardinia June 2013. Cassandra N. Trier, Tore O. Elgvin, Caroline Ø. Guldvog and G-P Sætre sampled Spanish sparrows in Kazakhstan April 2014, and A. Runemark, F. Eroukhmanoff and G-P Sætre sampled Spanish sparrows in Malta June 2014 (Fig. 1). The teams sampled one population in each of the latter three locations, all in all measuring and photographing 51 male Spanish sparrows. Finally, Richard I. Bailey, Lena Bache-Mathiesen and G-P Sætre sampled 53 male house sparrows in France and Switzerland, June 2014 (Fig. 1). We sampled two populations in France, Sales and Tempetay, and one population from Switzerland, Lignières. Coordinates for sample sites and the number of photographed individuals are summarized in Table S1. We caught the birds in mist nets and we made sure birds were handled carefully and released as soon as data was collected. All birds were measured for key morphometric traits, weighed and photographed, and a blood sample was taken for genetic analysis for each bird. However, only data extracted from the photographs are analyzed in this thesis. We had sampling permits from relevant local authorities for all sites.

Photography

We photographed the birds in a standardized light environment alongside a color checker (5.7 x 8.7 cm X-rite mini ColorChecker ®classic) to obtain comparable data (Fig. 2; for more details about the photographic setup, see Tesaker, 2014). We chose four male plumage traits, crown, cheek, back and rump, for color analysis as these differ between the parental species (Summers-Smith, 1988; Fig. 2). We used these to analyze patterns and constraints of male plumage in the Italian sparrow. We photographed sparrows from dorsal, ventral and lateral angles to cover the plumage areas of interest (Fig. 2). We photographed birds from a dorsal

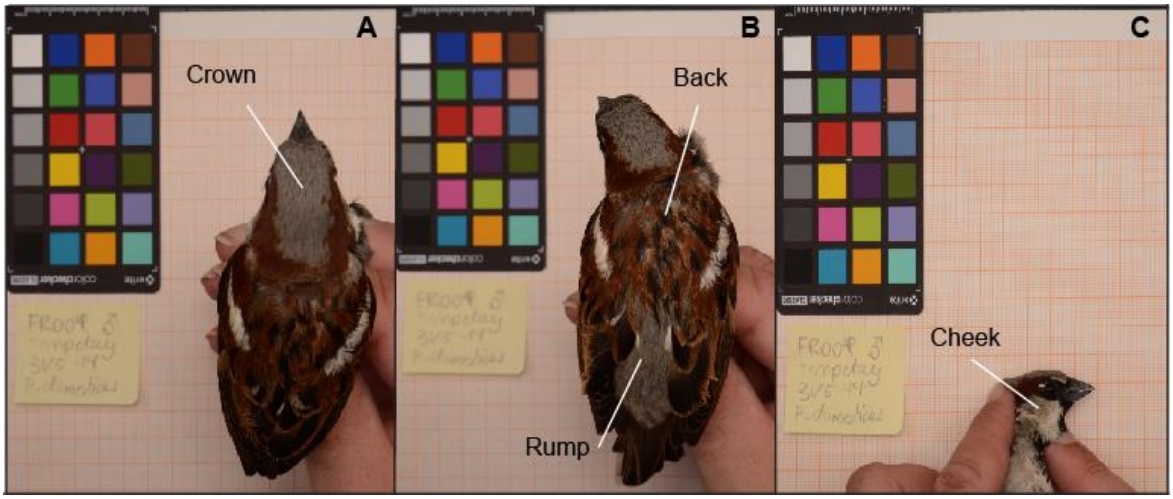


Figure 2. Examples of bird photos. The traits of interest were (a) crown, (b) back, (b) rump and (c) cheek. The ventral-view photo was not used in analysis and is not shown.

angle twice; once with the camera focused on the crown, and once more with its wings parted and the camera focusing on the rump (Fig. 2A-B). Many of the individuals were photographed twice from each angle. The second set of photos can be used to account for sampling error in the color analysis.

Color Quantification

The subtle nuances of color pattern variation make it a difficult phenotypic trait to quantify. A widely used measure of coloration is the amount of Red, Green and Blue (RGB) in the pixels of an image (Gerald et al., 2001). However, mean and standard deviation of these do not take spatial correlations between colors into account (Brydegaard et al., 2012). I quantified color using the Chromatic Spatial Variance Toolbox (Brydegaard et al., 2012; available at http://www.models.life.ku.dk/ChromatricSpatialVarianceToolbox) in MatLab R2013b (version 8.2.0.701; http://se.mathworks.com/products/matlab/). This method accounts for variance in spatial chromatic distributions; that color may change across a surface by patterns or patchiness. The toolbox uses the X-rite color checker in the image to standardize the coloration of each image to a uniform level, such that errors from slight variations between photographs will not be included in the analysis. It then normalizes the RGB data from the standard 0-255 scale (which is based on an adjustment to reflect human visual perception) to a 0-1 scale, proportional to light reflectance (0 = no reflectance, 1 = complete reflectance). After normalization, it allows the user to choose a specific target of interest and provides RGB data for each individual pixel in the target area (see Appendix I, Fig. S1 for chromaticity

plots of RGB for each respective trait per species, and Fig. S2-S5 for examples of individuals).

Before performing analytical statistics, I used the singular value decomposition (SVD) method in the Chromatic Spatial Variance toolbox on the RGB data, to reduce the between-individual color variation to a set of components (eigenvectors, eigenplanes or eigenfields, depending on dimensionality), each describing an aspect of the within-individual plumage color variation (Brydegaard et al., 2012; Fig. S6-S9). The Chromatic Spatial Variance Toolbox includes three scripts for running SVD on RGB datasets, one each for 1-, 2- and 3-dimensional SVD. SVD1D produces three 1D distributions using the amount of red, green and blue for each pixel. SVD2D uses the proportion of red and of green for each pixel to create a 2D histogram of color variation (Fig. S6-S9), and therefore measures chromatic color variation while removing variation in reflectance. SVD3D uses the 0-1 scale values of each of red, green and blue for each pixel, therefore incorporating both chromatic and reflectance variation in a 3D histogram of color variation. SVD2D performed best in correctly categorizing Skyros wall lizards (*Podarcis gaigeae*) into distinct groups based on color patterns, compared to the two other dimensionalities of SVD (Brydegaard et al., 2012), and it performed better than SVD1D for distinguishing brown from mixed crowns in Italian sparrows (Tesaker, 2014). However, variation in the cheek trait of the three sparrow species is dominated by variation in neutral colors (white-gray-black), which manifest primarily as differing brightness rather than different colors, accounted for by a reflectance intensity parameter in SVD3D, but not in SVD2D. Therefore, I used SVD3D for statistical analysis of cheek data, and SVD2D for all other traits.

I chose the number of eigenplanes from the SVD2D (or eigenfields for cheek color SVD3D) by comparing Cattell's break-point Scree Plot Test (Cattell, 1966) and Kaiser's eigenvalues-greater-than-one Information Criterion (Kaiser, 1960). I used a total of fifteen eigenplanes and eigenfields in further analysis: four Crown eigenplanes, three Back eigenplanes, five Rump eigenplanes and three Cheek eigenfields. The amount of variation for each species in the first three eigenplanes/fields per trait is shown in Figure S10.

I created a dataset containing only individuals with all four plumage traits sampled and used this in statistical analyses that did not allow missing data (Appendix II, Table S1). In addition, I made a dataset with all individuals, including those with missing data, and the SVD data from the second set of photos, to be used to account for measurement error where the analysis

method allowed this. 171 Italian sparrows, 51 Spanish sparrows and 53 house sparrows were included in this dataset, with repeated measures of at least one trait for 240 of these individuals (Table S1). Some individuals had their crown feathers raised in the photos, while others had them laid flat. As this could affect color quantification, I classified all birds with raised crown feathers as 'bushy' for crown photos.

The potential for a hybrid to form novel phenotypes

Plumage differentiation between species and between island populations of Italian sparrows

To determine whether there was variation in the plumage coloration between Italian sparrows and the parental species, and if it varied between the islands Corsica, Crete and Sicily, I ran a canonical variates analyses (CVA) (Campbell and Atchley, 1981) based on Mahalanobis distances (Hotelling, 1936, Mahalanobis, 1936) , with the Morpho package in R (version 3.1.3; download available at http://www.r-project.org/) (Schlager, 2013). I ran CVA on each plumage trait individually and all combined, once with species as a grouping factor and once with species and also with Italian sparrows further grouped by island (hence five groups). To infer which species and islands differed significantly from each other, I ran a permutation test with Mahalanobis distances in the Morpho package. These analyses indicate the extent to which Italian sparrow plumage trait values have intermediate means, and low variances such that all trait values fall between the parents, or have unique trait values or trait combinations (transgression or mosaicism). The latter, based on five groups, is also informative of differences in intermediacy, transgression and mosaicism between islands. To investigate if the Italian sparrow was transgressive in any of the study traits I classified Italian sparrow plumage as intermediate between the parental species if their center point (i.e. means along CV axes 1 and 2) fell between those of house and Spanish sparrows on both CV axes. Center points falling outside those of the parents on either CV axis were classified as transgressive if Italian sparrows were also significantly differentiated from both parents. To identify transgression on an individual level, caused either by increased variation in the hybrid species or by transgressive center points, I looked for hybrid individuals with CV scores outside the range of all parental individuals, regardless of center point position.

I also used Multivariate Analysis of Variance (MANOVA) as a second test of differentiation, through the default command in R. To infer the significance of a factor, an F-test is commonly used in univariate data, but is suboptimal in multivariate cases. Here, I used the Pillai-Bartlett Trace statistic (Pillai, 1955), see also (Olson, 1976).

I ran a MANOVA for all species and all traits combined, and then one for each of the four traits individually. To compare islands, I ran MANOVA models for the Italian sparrow island populations of Corsica, Crete and Sicily, one for all traits combined, and one for each of the four traits individually. Bushy-crown was added as a random effect factor in models concerning all trait plumage and for models for crown plumage. To infer which MANOVA model best explained the variation for each analysis, I used Akaike's Information Criterion (AIC) (Akaike, 1974).

While the CVA analyses described above will reveal general patterns of intermediacy, transgression and mosaicism in hybrid plumage coloration, it is also important to know where the Italian sparrow plumage falls along the primary axis of parental differentiation, as this is expected to be the axis where early generation hybrids have the largest increase in their ability to respond to selection compared to the parent species. The objectives were to estimate whether Italian sparrows were more similar to one parent or the other, whether they were transgressive along this axis (in mean or variance), the extent to which this differed between plumage traits, and the extent to which islands differed in their pattern of similarity to the parents along this axis. To see if the hybrid species is more or less similar to one parental species or the other, and find out whether the amount of resemblance varied between the island populations, I used a linear discriminant analysis (LDA) (Fisher, 1936) from the MASS package in R (Venables and Ripley, 2002) to identify the axis of differentiation between house and Spanish sparrows, and subsequently calculate a score along this axis for every individual. LDA is closely related to CVA in that it finds the axes maximizing the variance between a group of predetermined classes (Fisher, 1936), and can similarly be regarded as maximizing between-group distance in units of conditional evolvability. LDA was carried out on each trait individually, and resulting values were scaled so that 0 = house sparrow mean discriminant score and 1 = Spanish sparrow mean.

The effects of diversity and constraints in a hybrid species

Measures of evolutionary potential

To understand the evolutionary potential of Italian sparrows and their parent species I needed to estimate trait variability and constraints at different scales - within and between species, islands (for Italian sparrows) and populations - and to compare these quantities among plumage traits. Several of the statistical methods I described above provide related information on evolutionary potential, and I summarize their relationships here.

From a multivariate matrix of phenotypic trait variances and covariances, or P-matrix, one can implement analyses to infer the evolutionary potential of a population, including evolvability statistics (Hansen and Houle, 2008, Roff 2012). Evolvability is a measure of how capable a population is to respond to selection in the absence of constraints caused by selection on correlated traits, while conditional evolvability is the ability for a trait to respond to selection if its correlated traits are not allowed to change (Hansen and Houle, 2008). Autonomy, the conditional evolvability divided by the evolvability, is the proportion of evolvability that is independent from potentially constraining trait correlations (Hansen and Houle, 2008). These statistics aid in inferring the full potential for a population to evolve. In addition, one can determine whether two populations will, under selection pressure, follow different evolutionary trajectories by comparing the response of their P-matrices to a multitude of random selection gradients, a method known as random skewers (Cheverud and Marroig, 2007).

Both evolvability and conditional evolvability are in units of trait variance. The Mahalanobis distances (as described previously) are also on the same scale, and represent measures of distance between two multivariate values (for example, species means for a set of traits) in units of the conditional evolvability along that axis. Canonical Variates Analysis maximizes the between- versus within-group Mahalanobis distance. This is therefore the axis of maximum between- versus within-group conditional evolvability, and hence the axis along which evolution between group means should be most constrained and most difficult to accomplish.

Average evolvability represents the average variance across all directions in multivariate trait space. Total variance is then the average evolvability multiplied by the number of traits. Variance components analysis calculates the same total variance and divides this up among

the effects of predictors, such as between- versus within-species variance. Hence several of the analyses carried out below provide related information that can be thought of in terms of evolutionary potential.

The extent of variation explained at the island, population and species level

The extent to which trait variation is distributed within and among populations may differ between a hybrid species and its parents, and to investigate how much of the variation is explained at the between-species, between-island, between-population and within-population levels, I used variance decomposition. A variance decomposition analysis can be used to infer the magnitude of effect each factor has on the response variables, and consequently to estimate the proportion of the total variance explained by each factor. More within-population variation would indicate greater current potential to respond to selection, while more between-population variation would indicate greater potential for further diversification and speciation. To obtain the variance components of the factors (species, island, population, individual, and measurement error from repeated measures and from crown bushiness), I employed Bayesian Markov chain Monte Carlo Generalized Linear Mixed Model analysis using the MCMCglmm package in R (Hadfield, 2010). This is a highly flexible package that allows the user to incorporate repeated measures and random effects into the model, and to estimate the posterior mean and degree of confidence (upper and lower highest posterior density, HPD) for the variance and covariance matrix of multivariate responses, with respect to each factor and the residuals. For fixed effects in the MCMCglmm analyses, default priors (zero-mean diagonal matrix with large variances; Hadfield 2010) and Gaussian error structures were used. For variance and covariance matrices for random effects and residuals, I used inverse-Wishart distributed priors (Hadfield, 2010), specifying V (the expected (co)variance matrix at the limit) and n (degree of freedom parameter). Testing indicated that accurate estimates of the (co)variance matrix of response variables were attained by setting V to be a diagonal matrix (hence with zero covariance) with variances one order of magnitude lower than expected (the 'expected' values were calculated by making standard covariance matrices from the data in R), and setting the quantity n to the number of responses + 1.

The fitted model took the following general form (written in R code):

```
fixed = trait – 1,

random = ~ idh(trait):species + idh(trait):population + us(trait):individual,

rcov = ~ us(trait) :units,
```

where 'trait' indicates multiple response variables, 'idh' specifies estimation of trait variances with covariance assumed zero, and 'us' specifies estimation of the full covariance matrix. The variance components for each factor were calculated as the sum of the posterior mean variance estimates across all responses. This model was also run for Italian sparrows-only, with 'species' replaced by 'island', and for Spanish sparrows- and house sparrows-only and each island individually with 'population' and 'individual' as random effects. Model comparison using DIC (Hadfield 2010) indicated that estimating between-group covariance (i.e. employing 'us' rather than 'idh') did not improve the fit of the model, and hence I only estimated the full covariance matrix for individual-level variation and for the residuals, which represented measurement error from repeated measures.

Variance components analysis was carried out on each plumage trait individually, and also on eigenplane/field 1 for each trait, in order to combine traits, as MCMCglmm failed to run when using all 16 eigenplanes/fields from all four traits. Analyses that involved crown also included the random factor 'bushy' to further reduce the impact of measurement error. Each model was run with 50 000 iterations, a burnin at 8 000 and a thin at 25. To infer the significance of differences in variance component proportions between groups, I calculated the 97.5% and 2.5% quantiles of the variance components proportions per trait (Appendix II, Table S2).

Differences in average evolvability between species and populations

To find out how capable the Italian sparrow is at responding to random selection gradients compared to its parental species, and whether islands differed in the ability to respond to selection, I calculated average evolvability and average conditional evolvability (Hansen and Houle 2008) for each species and island. I used the evolvability package in R, which has a multitude of tools for estimating the evolvability-related measures described in Hansen and Houle (2008), and includes adaptations for use with the MCMCglmm package (Bolstad et al., 2014). Evolvability statistics can hence be estimated along with measures of upper and lower

confidence limits, using the full posterior distribution of the MCMCglmm individual-level covariance matrix. For these analyses I used only one measurement per individual, so that the residuals represented the between-individual covariance matrix. "Bushy" was used as a random effect in models including crown. Any statistical analysis that uses the variance, and off-diagonals of the covariance matrix, are susceptible to be skewed by low sample sizes (Whitlock and Schluter, 2009). To improve comparability of evolvability measures among islands and species, I therefore subset the datasets for each species and island, and resampled without replacement for one hundred iterations per trait to match the smallest sample size of each group; for species, Spanish sparrows had the smallest sample size of 51 individuals, and for islands, Crete had the smallest sample size of 42.

I calculated average evolvability statistics for all plumage traits, as well as crown, back, rump and cheek separately. I used MCMCglmm to create the posterior distribution of the covariance matrix (P-matrix), with which to then calculate all evolvability statistics per sub-sample, except when calculating evolvability for all traits simultaneously, where a covariance matrix was extracted directly from the dataset. Before running MCMCglmm, I multiplied all values by 100 to increase the variance and improve MCMCglmm model-fitting; this was also done on the P-matrix for all traits, so that the scale of evolvability measures matched those derived from the MCMCglmm covariance matrices. MCMCglmm was run for 25000 iterations, with a burnin of 5000 and thinning interval of 20, producing 1000 posterior covariance matrices. All evolvability statistics from the full posterior distribution of the MCMCglmm for each of the random subsamples were stored. Therefore for each trait, the 100k (100 random subsamples x 1000 values in the posterior distribution) evolvability estimates incorporated both error variation from random subsampling and sampling error from the Bayesian posterior distribution (except the smallest sample in any comparison, for which the 100k values did not include subsampling error). These results were used to calculate the median and 2.5% and 97.5% quantiles of the error distribution for all evolvability statistics (Appendix II, Table S3-S7). Overlap of upper and lower quantiles among groups with standardized sampling can be considered as a significance test.

Alongside average evolvability and average conditional evolvability I calculated average autonomy to infer if the species and islands varied in the degree to which evolvability is affected by correlations between plumage variables. The formulae used for average autonomy and integration are approximations (Hansen and Houle, 2008). Therefore to make these

calculations more accurate, I made one thousand random unit vectors to act as selection gradients - as recommended within the evolvability package - calculated evolvability statistics along each of these gradients and calculated the average autonomy, integration and conditional evolvability from these results for each of the six subsamples.

The extent of constraints along the parental axis of differentiation

I investigated whether the Italian sparrow was more or less evolvable along the parental axis of differentiation than the house and Spanish sparrow, and whether this differed among the islands, by rescaling the discriminant axis between parental means to a unit vector (Schluter, 1996) and calculating evolvability along that direction, using the evolvabilityBeta option from the evolvability package. All other details were as described in the calculating average evolvability section.

The similarity of species' and islands' response to selection

To investigate how similarly to the parental species the Italian sparrow responds to selection, and whether this varied between the island populations, Corsica, Crete and Sicily, I used the random skewers method (Cheverud & Marroig, 2007). This was implemented with the phytools package in R (Revell, 2012). Results from random skewers may be skewed by low sample sizes, which particularly affect covariances between variables. Therefore, I calculated the random skewers correlation and p-value between each pair of covariance matrices (representing either the 3 species, or 5 groups: house sparrow, Spanish sparrow, Corsica, Crete and Sicily) for each of one hundred sub-samples matching the smallest sample size, using the "unifcorrmat" argument as recommended by the phytools package to obtain a more accurate estimate of p-values. I recorded the median correlation from the 100 subsamples for each pairwise species or island comparison. I then created a pairwise distance matrix from these median correlations, and ran a principle coordinates analysis (PCoA) (Torgerson, 1958, Gower, 1966) on the distance matrix in order to visualize the results. The resulting scores can be considered as an n-dimensional map of distances between groups. A covariance matrix was made for each group from the full set of 16 plumage eigenplanes/fields.

Results

The potential for a hybrid to form novel phenotypes

Plumage differentiation between sparrow species

The three *Passer* sparrow species differed significantly for all plumage traits, individually and combined, and for most pairwise species comparisons as revealed by CVA and MANOVA (Table 1, Table 2, Fig. 3). In general, house sparrows had gray crowns, brown and black backs, solid gray rumps and grayish-white cheeks (Fig. 3; Appendix I, Fig. S1-S5); Spanish sparrows displayed a chestnut colored crown, black and white back, black-spotted rump and bright white cheeks (Fig. 3; Fig. S1-S5); while the Italian sparrow showed chestnut colored crowns, black, white and brown backs, grayish brown or black-spotted rumps and bright white cheeks (Fig. 3; Fig. S1-S5). House and Spanish sparrows formed distinct, non-overlapping clusters for combined plumage traits and for crown color, and also showed very little overlap in back or rump. Cheek color was the only trait where the two parental species formed overlapping clusters (Fig. 3), but they were nevertheless highly significantly differentiated (Table 2).

Table 1. Species and island plumage differentiation for all traits (crown, back, rump and cheek) combined, from MANOVA analysis on plumage eigenplanes/fields.

Group	Trait	Model[1]	Df[2]	V[3]	F[4]	p-Value
Species	All Traits	Population	14, 250	4.173	6.58	<2.2e-16
	Crown	Population	14, 255	1.769	14.44	<2.2e-16
	Back	Population	14, 250	1.428	16.23	<2.2e-16
	Rump	Population	14, 250	1.754	9.65	<2.2e-16
	Cheek	Population	14, 250	1.005	5.99	<2.2e-16
Islands	All traits	Population	8,152	2.522	4.14	<2.2e-16
	Crown	Population	8,157	0.848	5.28	<2.2e-16
	Back	Population	8,152	0.898	8.12	<2.2e-16
	Rump	Population	8,152	1.202	6.01	<2.2e-16
	Cheek	Population	8,152	0.748	4.37	<2.2e-16

[1]Presented models selected by AIC (see Appendix II, Table S8). Due to the nested nature of these factors, the population model includes variation from island and species levels.
[2]Degrees of freedom for: fixed effect, residuals
[3]Pillai-Bartlett Trace test statistic (Pillai, 1955)
[4]Approximate F-value

For combined traits, all three species were significantly differentiated (Table 2, Fig. 3). Italian sparrows had a transgressive center point; e.g., a center point falling outside those of the parents on both CV axex while both parents were significantly different from the Italian sparrow. This indicates either transgressive individual plumage color values, or mosaicism of plumage traits. However, there were very few fully transgressive individuals, suggesting that the transgression was mainly caused by mosaicism.

Italian sparrows had crowns matching those of Spanish sparrows in both center point and variation of CV scores (Table 2, Fig. 3). Their cheek plumage also closely matched that of Spanish sparrows, and was only significantly differentiated from house sparrows. For cheek plumage, all three species exhibit high variability; in contrast, there was little variation in crown color. Neither trait showed evidence of transgression. For the back and rump, Italian sparrows had transgressive trait means and displayed wider between-individual variation than both house and Spanish sparrows (Table 2; Fig. 3). However, very few individuals had trait values outside the range of the parents for the rump, while many individuals were transgressive for back plumage. Hence, the back was the most transgressive plumage trait in the hybrid Italian sparrow.

Table 2. Differences between sparrow species in each plumage trait shown by Mahalanobis distances and p-values (in parentheses) from a permutation test of a canonical variates analysis.

Trait	Species	House	Italian
All Traits	Italian	8.836 (0.001)	
	Spanish	9.521 (0.001)	3.179 (0.001)
Crown	Italian	8.505 (0.001)	
	Spanish	8.482 (0.001)	0.125 (0.992)
Back	Italian	1.164 (0.001)	
	Spanish	2.159 (0.001)	2.547 (0.001)
Rump	Italian	2.062 (0.001)	
	Spanish	3.122 (0.001)	1.706 (0.001)
Cheek	Italian	1.631 (0.001)	
	Spanish	1.686 (0.001)	0.430 (0.088)

Mahalanobis values shown as: distance (MD), probability (Mp)
The minimum possible p value was 0.001, given the 1000 permutations used

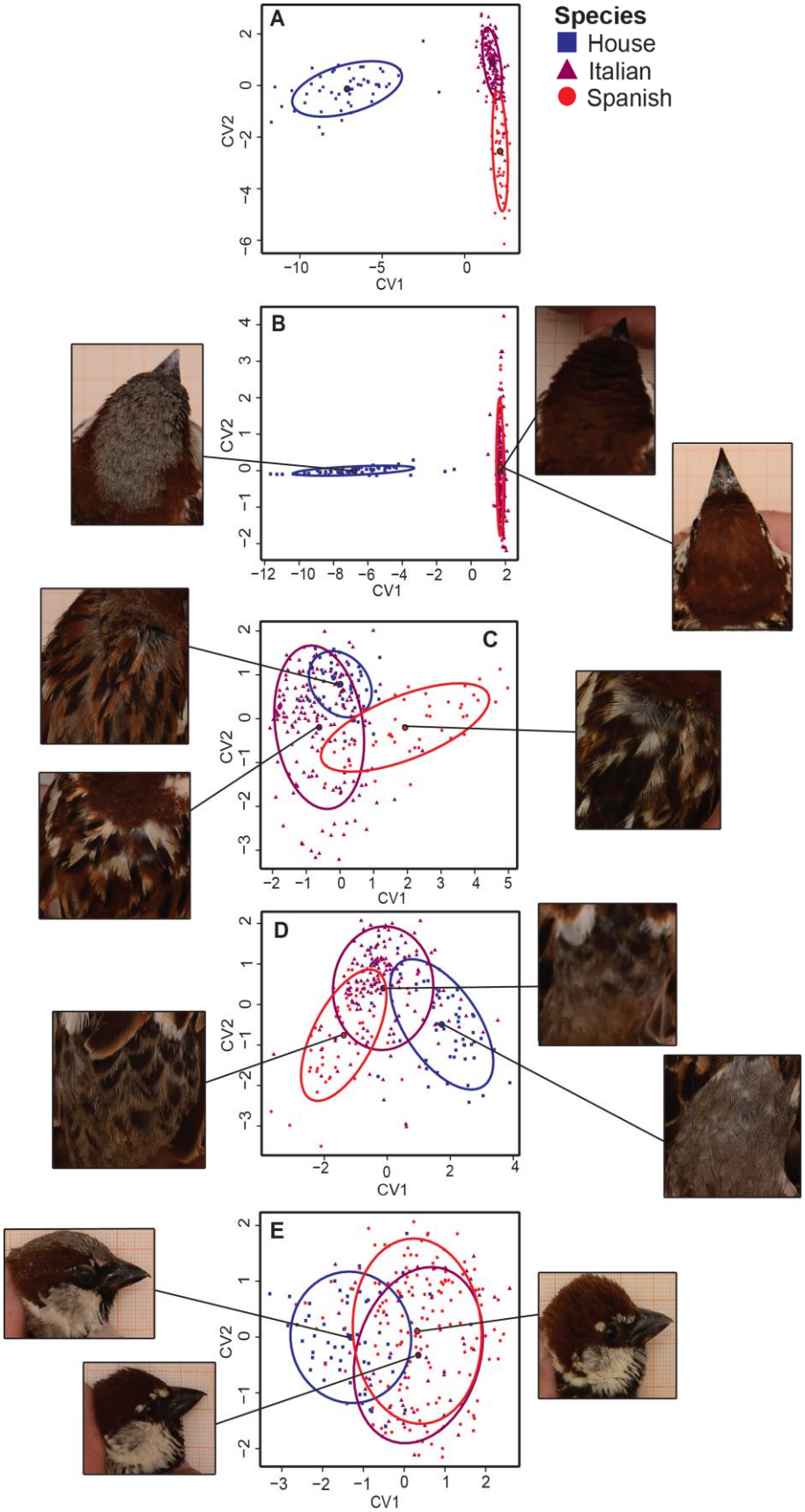


Figure 3. Species differentiation in plumage color traits. Variation is shown in all traits (A), crown (B), back (C), rump (D) and cheek color (E). Data is derived from a canonical variates analysis with species as a grouping factor on SVD2D data (SVD3D for cheek). Ellipses show 80% quantiles, and center point indicates the mean for each species. Each photo represents an individual with a score which is close to the average of its species.

Island contingency in hybrid plumage

I found highly significant plumage color differences between island populations of Italian sparrows for all plumage traits, both combined and individually (Table 1). The Italian sparrows of Corsica and Crete were more similar to each other than to those of Sicily for all traits except crown color, but were nevertheless significantly differentiated for all traits except rump (Table 3, Fig. 4). There were more transgressive individuals for combined traits when splitting the islands than when lumping them together (compare Fig. 3A and Fig. 4A). Plumage traits on Corsica and Crete individuals were either intermediate between the parents or similar to one parent or the other, while Crete individuals had relatively high variability. However, all islands were significantly differentiated from both parents for all traits except crown, which was not differentiated from Spanish sparrows. Sicily displayed the most transgression for back and rump color, and was the most diverged from the parent species in these two traits (Table 3; Fig. 5E, G).

Table 3. Differences between house and Spanish sparrows and between three island populations of Italian sparrow in each plumage trait. Mahalanobis distances and p-values (in parentheses) are derived from a permutation test of a canonical variates analysis.

Trait	Species/Island	House	Corsica	Crete	Sicily
All Traits	Corsica	8.857 (0.001)			
	Crete	8.885 (0.001)	2.696 (0.001)		
	Sicily	9.764 0.001)	4.642 (0.001)	4.261 (0.001)	
	Spanish	9.642 (0.001)	3.689 (0.001)	4.328 (0.001)	4.048 (0.001)
Crown	Corsica	8.509 (0.001)			
	Crete	8.625 (0.001)	2.278 (0.002)		
	Sicily	8.469 (0.001)	1.292 (0.070)	1.072 (0.205)	
	Spanish	8.453 (0.001)	1.146 (0.095)	1.158 (0.162)	0.186 (0.981)
Back	Corsica	1.049 (0.001)			
	Crete	0.806 (0.024)	0.884 (0.006)		
	Sicily	2.898 (0.001)	2.306 (0.001)	2.677 (0.001)	
	Spanish	2.431 (0.001)	2.850 (0.001)	2.900 (0.001)	2.736 (0.001)
Rump	Corsica	2.180 (0.001)			
	Crete	1.914 (0.001)	0.783 (0.066)		
	Sicily	3.488 (0.001)	3.397 (0.001)	3.010 (0.001)	
	Spanish	3.174 (0.001)	1.859 (0.001)	2.240 (0.001)	2.858 (0.001)
Cheek	Corsica	1.874 (0.001)			
	Crete	1.157 (0.001)	0.728 (0.009)		
	Sicily	2.103 (0.001)	1.271 (0.001)	1.341 (0.001)	
	Spanish	1.739 (0.001)	0.698 (0.003)	0.826 (0.001)	0.849 (0.002)

Mahalanobis values shown as: distance (MD), probability (Mp)
The minimum possible p value was 0.001, given the 1000 permutations used

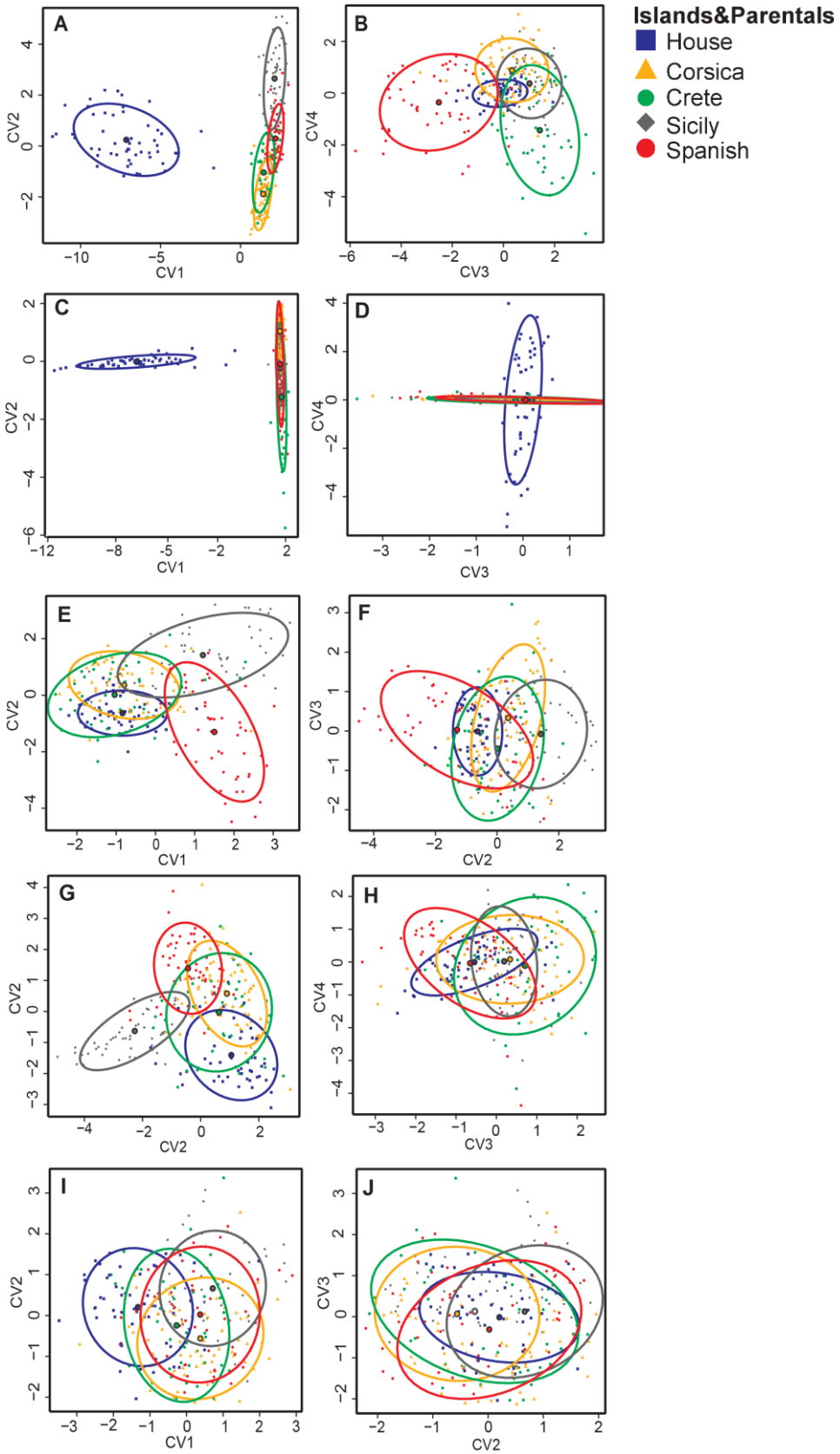


Figure 4. CVA plots with illustrate differentiation in plumage color between islands and parent species for each trait. Variation is shown for all traits (A-B), crown (C-D), back, (E-F) rump, (G-H) and cheek (I-J). Data is derived from a canonical variates analysis with the islands and parentals as a grouping factor on SVD2D data (SVD3D for cheek). Ellipses show 80% quantiles, and center point indicates the mean for each group.

Linear discriminant axis scores along the axis of parental differentiation further indicated that Italian sparrows are mosaic in their plumage pattern, and showed among-island contingency in their inheritance of parental plumage traits (Fig. 5). While the island birds all had Spanish-like crown color along this axis, they differed considerably in back color in particular, with Sicily resembling Spanish sparrows and Corsica and Crete resembling house sparrows (Fig. 5B). The island populations were generally more intermediate between the parents in rump and cheek color, but with variation both between islands and between populations within islands (Fig. 5C-D). High variability of some traits suggested there may be some transgression along the axis of parental differentiation.

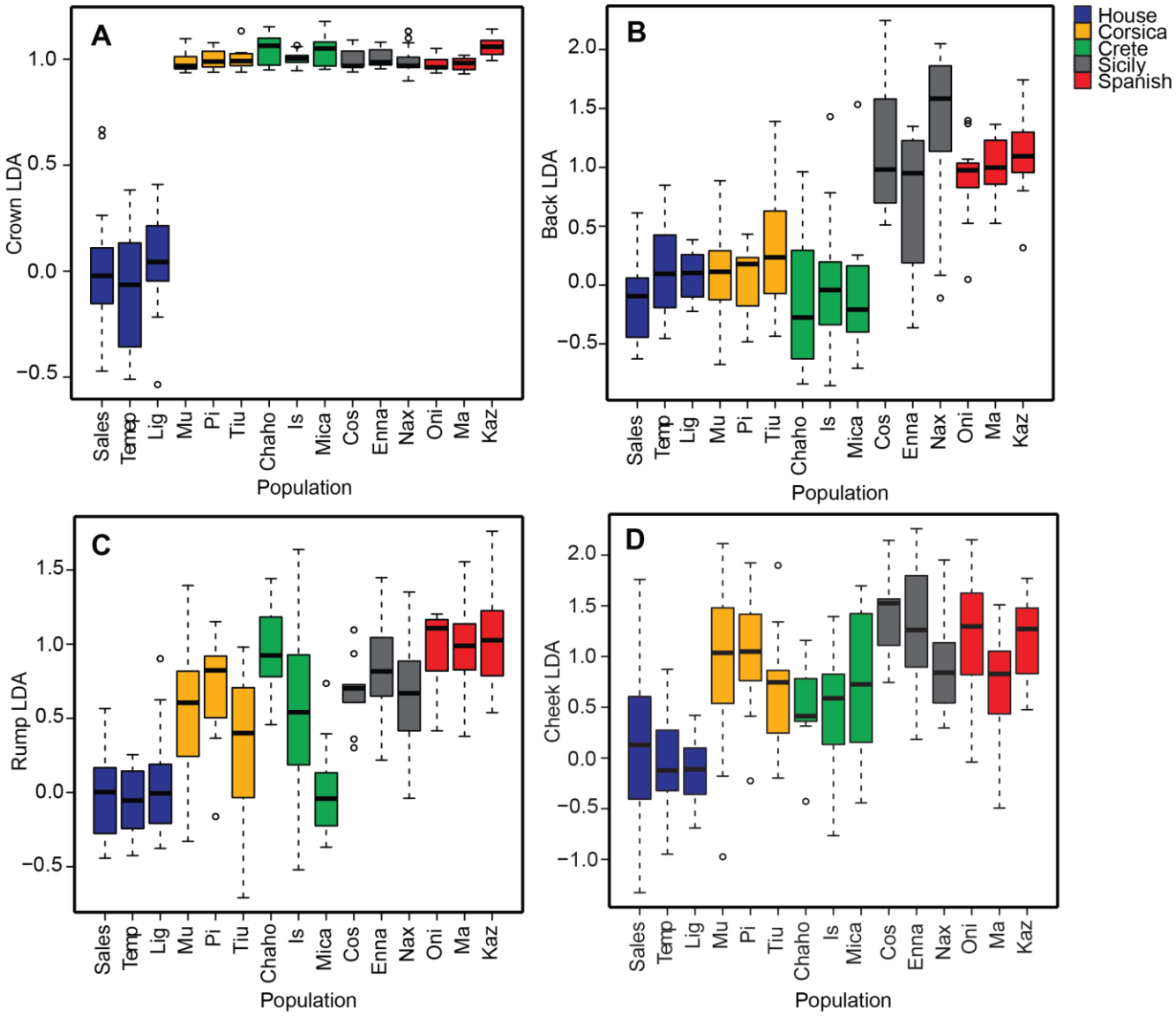
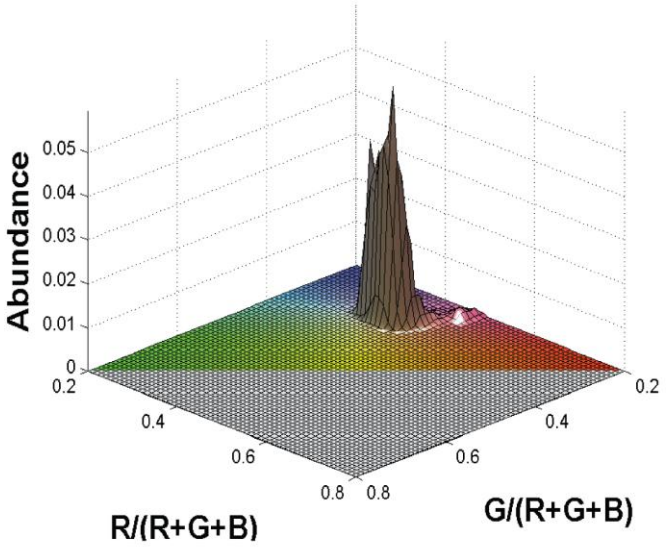


Figure 5. Linear discriminant scores of individual populations of all three species along the axis of parental differentiation. Variation is shown for crown (A) back (B), rump (C) and cheek (D). The parental range is scaled to 0 for the house sparrow mean and 1 for the Spanish sparrow mean.

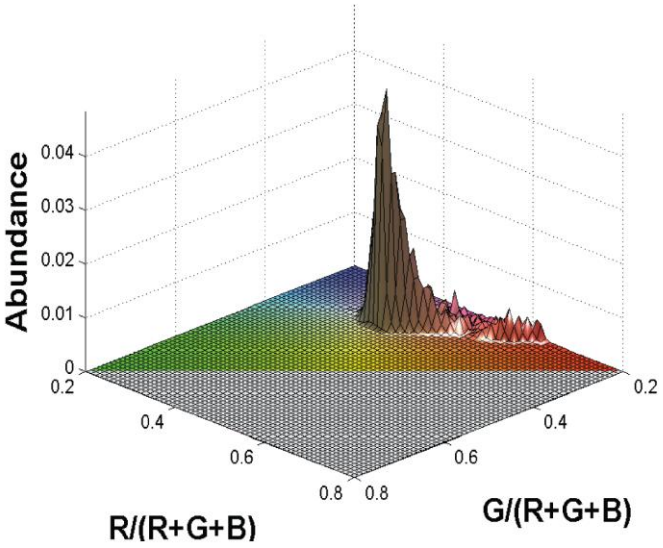
Examples of transgression in plumage traits

Several Italian sparrows displayed values outside of the parental range, particularly for back and rump color (Table 2; Fig. 3-5). Chromaticity plots show the back color differences between a transgressive Italian sparrow and a house and Spanish sparrow (Fig. 6): while the Spanish sparrow had a black and white back, the house sparrow a brown, gray and black back, the transgressive Italian sparrow had patchy back plumage with intermittent brown and light brown spots (Fig. 6). In general, it also appeared redder in chromaticity than either of the parentals. The transgressive Italian sparrow rump had a solid pattern with a brown-tinted gray color, unlike the patchy, black and gray colored rump of Spanish sparrows and the completely solid gray rump of house sparrows (Fig. 7).

A



B



C

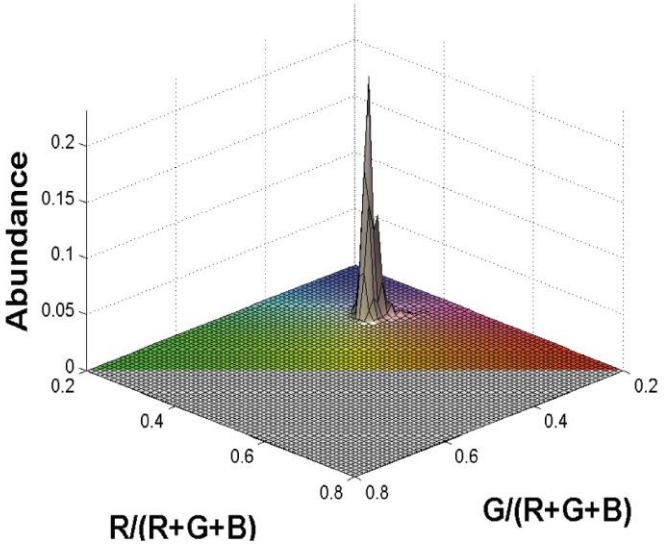
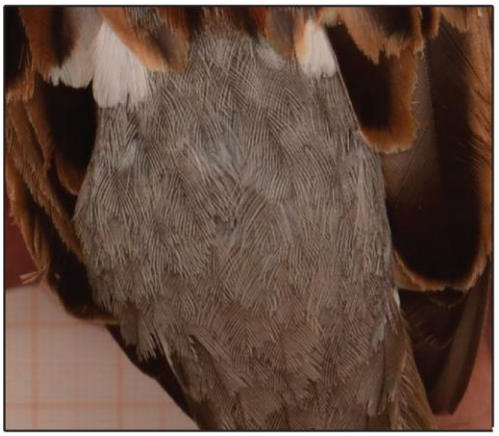
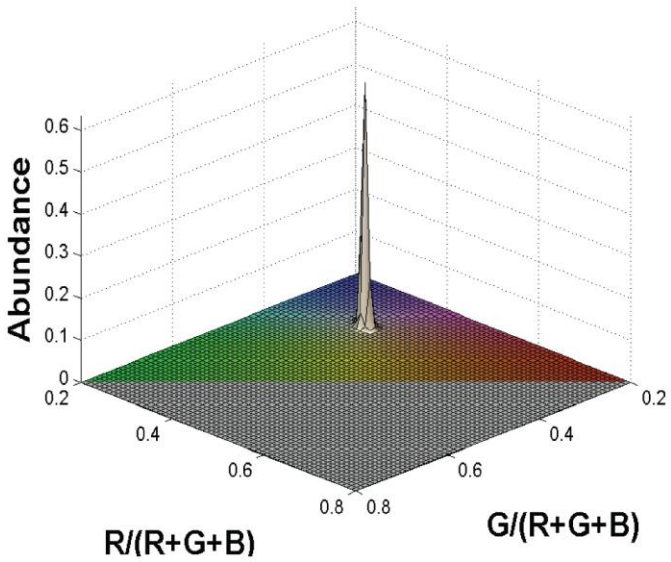


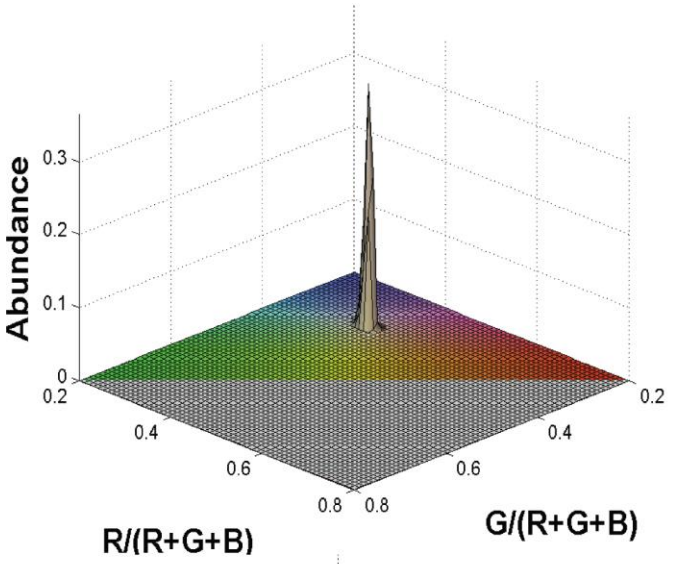
Figure 6. The differences between back color in a transgressive Italian sparrow and in the two parental species. Chromaticity histograms represent the back color in the photo to the left, for a house sparrow (A), a transgressive Italian sparrow (B) and a Spanish sparrow (C).

A





B



C

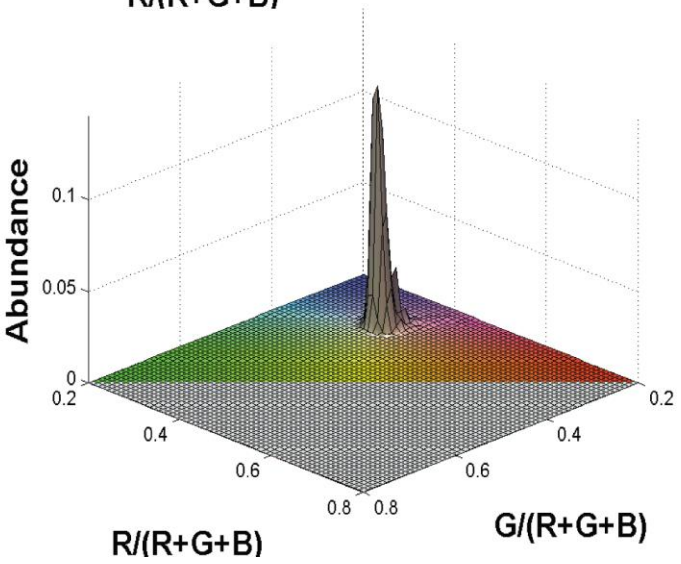


Figure 7. The differences between rump color in a transgressive Italian sparrow and in the two parental species. Chromaticity histograms represent the rump color of the photo to the left, for a house sparrow (A), a transgressive Italian sparrow (B) and a Spanish sparrow (C). Note that the mean chromaticity distribution is slightly browner and wider for the Italian and Spanish sparrow than the house sparrow, indicating larger variation in color for these two species.

The effects of diversity and constraints in a hybrid species

The extent of variation explained at the island, population and species level

The variance decomposition revealed that, across species, the highest proportion of variation was found at the species level for combined plumage traits, crown color and rump color, but the proportion explained by each factor level varied between traits (Fig. 8A-B). The individual level explained the highest proportion of variation in back and cheek color.

The proportion of variation explained at the species level was significantly higher in crown color than in back and cheek color, while rump color did not differ significantly from any trait (Appendix II, Table S2).

I found limited evidence for contingencies between islands, as the island level explained relatively small amounts of variation compared to the population and individual level for crown, back and cheek plumage (Fig. 8C-D). However, the island factor accounted for 32.5% of the variation in rump color. The proportion of variation explained by the island level was, however, not significantly different between any traits (Table S2).

The individual level explained most of the variation in house sparrows for each of the traits (Fig. 8E-F). Spanish sparrows shared a similar pattern to house sparrows for back and rump plumage, but the population level was more important for Spanish sparrows in crown and cheek plumage, and in fact, the proportion of individual level variation in crown color was significantly lower from that of house sparrows (Table S2).

The amount of variation explained at the population- and individual level varied between Corsica, Crete and Sicily (Fig. 9). The individual level explained the majority of the variation in all traits for all islands. There was also a high proportion of between-population variation in back color in Crete (Fig. 9). For Sicily, more of the variation was found at the population level in crown, rump and cheek color compared to Crete and Corsica (Fig. 9E-F). However, none of the islands were significantly different from each other in the proportion variation explained by each factor level (Table S2).

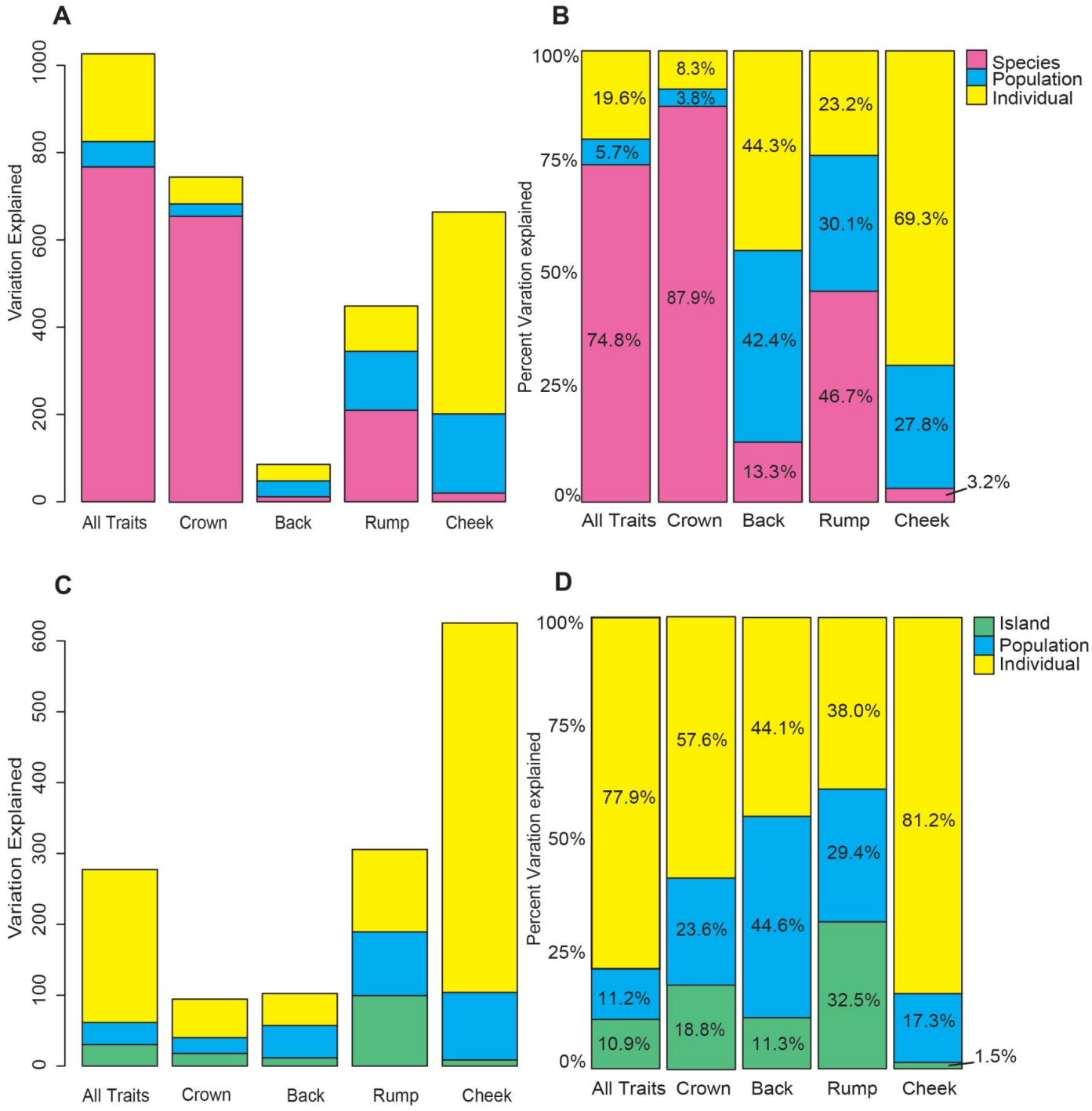


Figure 8. The plumage color variance components explained at the species, island, population and individual level. Plots are continued on page 31. The amount and percentage explained by each factor level is shown between all species (A-B) and Italian sparrows (C-D) on page 30 and in house sparrows (E-F) and Spanish sparrows (G-H) on page 31. 'All Traits' includes only the first eigenplane/field from each trait.

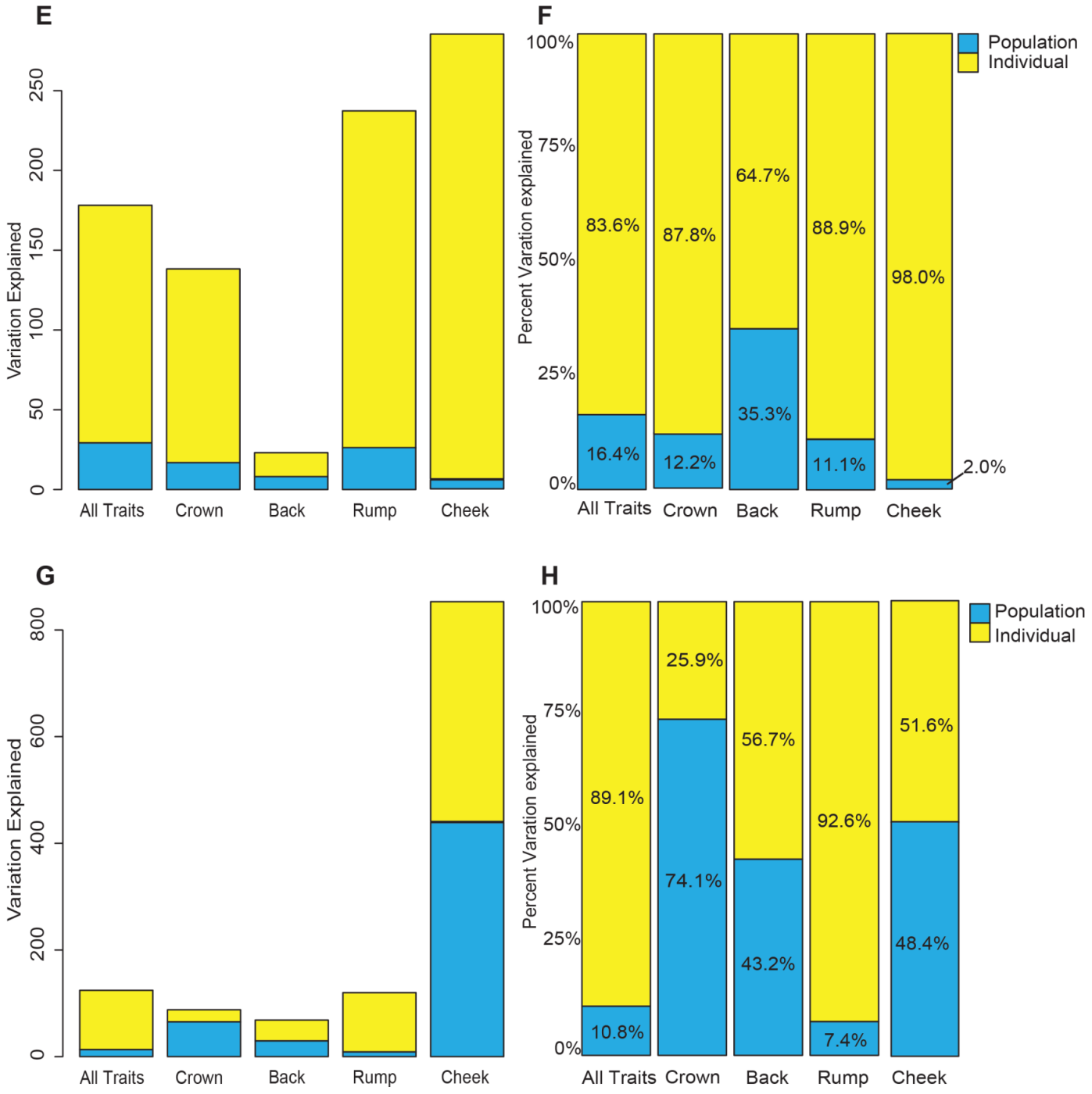


Figure 8. Continued.

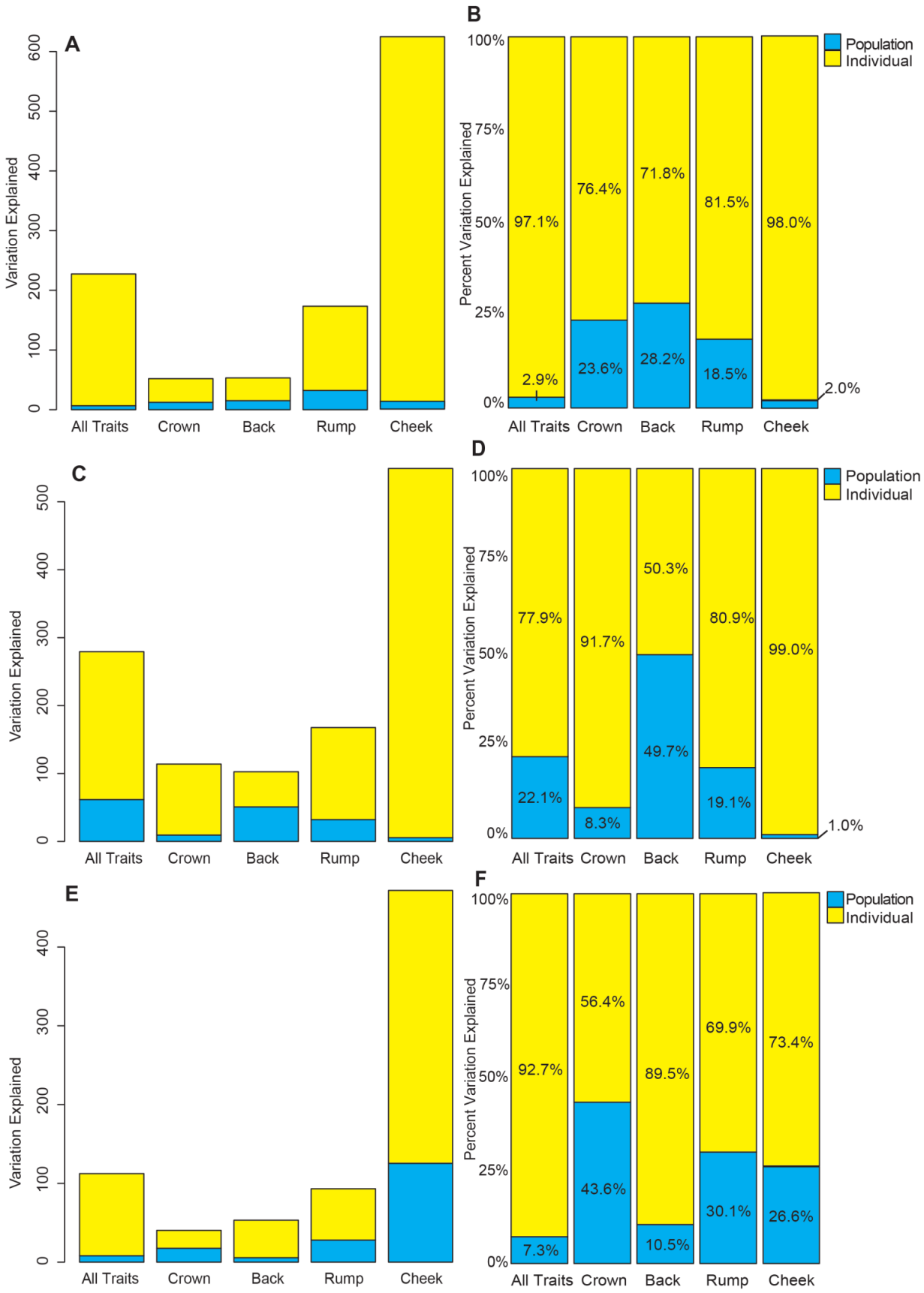


Figure 9. The plumage color variance components explained at the population and individual level for each island population of Italian sparrows. The amount and percentage explained by each factor level is shown for Corsica (A-B), Crete (C-D) and Sicily (E-F). 'All Traits' includes only the first eigenplane/field from each trait.

Differences in average evolvability statistics between species and populations

I decided that crown color should be removed from combined-trait evolvability measures and plots. This was because of very low amounts of within-species variation in comparison to between-species variation in crown color (Fig. 10; but for comparisons of plots including all traits and plots without crown, see Appendix I, Fig. S11) strongly affected the evolvability statistics when all traits were considered simultaneously.

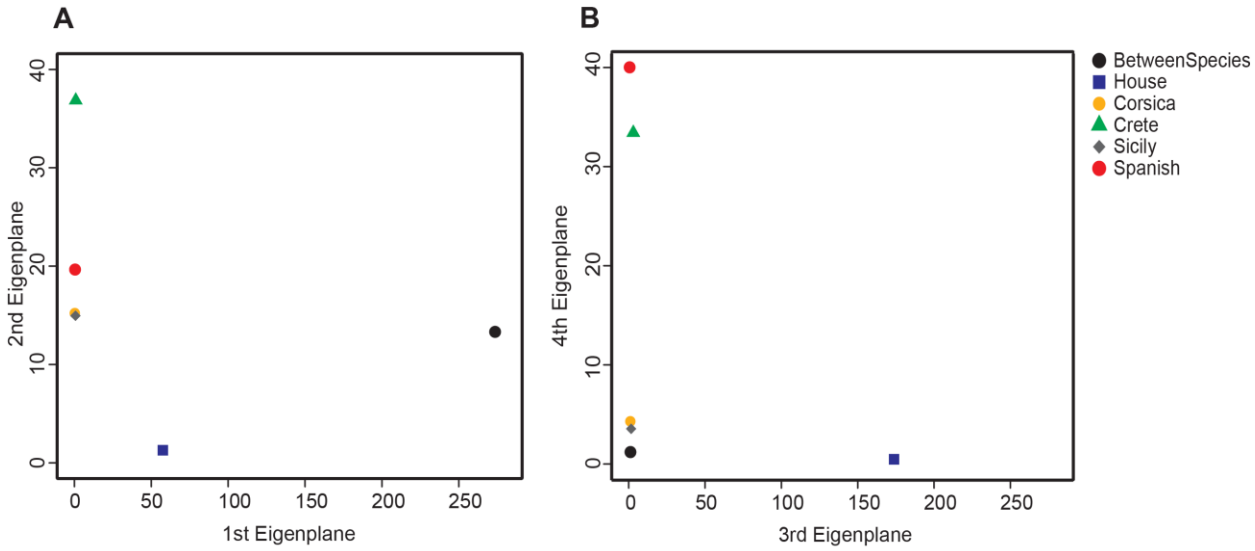


Figure 10. Crown color variation within and between species and islands. Variances are shown in each of the significant crown eigenplanes. Eigenplane 1 and 2 are illustrated in A) and Eigenplane 3 and 4 in B).

For the most part, Italian sparrows would be significantly more capable of responding to selection than house and Spanish sparrows, according to the upper and lower quantiles of average evolvability ($e_{\bar{y}}$) for all plumage traits (Fig. 11; Appendix II, Table S3).

Crown was the only trait where Italian sparrows were less evolvable than one of the two parentals: the house sparrow crown color is significantly more evolvable than the Italian and Spanish crown color (Fig. 11B; Appendix II, Table S4). However, despite high average evolvability in crown color for the house sparrow, its average conditional evolvability ($c_{\bar{y}}$) was very small, not significantly different from the other two species, meaning that crown had little potential to evolve under selection if its constraining traits did not change (Fig. 11B; Table S4).

Italian sparrows had the highest potential to respond to selection in back color (Fig. 11C). The amount of evolvability and conditional evolvability in back color was significantly lower for house sparrows than for the two other species (Fig 11C; Appendix II, Table S5). Italian sparrows were also the most evolvable species for rump color, for which house sparrows were the most evolvable parent species (Fig. 11D, Appendix II, Table S6). For cheek color, house sparrows had relatively low average evolvability compared to the other species, while the ability to respond to selection was relatively similar between Italian and Spanish sparrows (Fig. 11E, Appendix II, Table S7).

Island populations of Italian sparrows varied in how responsive to selection they would be, and Crete was, in general, more evolvable than the other two islands for all plumage traits (Fig. 12A). This is consistent with previous results from a CVA (Fig. 4) and variance decomposition (Fig. 9). Back plumage color was the only trait where Crete did not have the highest evolvability, but even here, Crete had a higher average conditional evolvability than the other two islands (Fig.12C). Crete was significantly more evolvable in crown color than the other two island populations (Fig. 12B, Table S4). Sicily had the lowest evolvability in rump plumage color and cheek color, while Corsica had the lowest evolvability for crown color, and Sicily had the lowest evolvability, significantly lower than Crete, when considering back, rump and cheek plumage combined (Fig. 12A, Table S3).

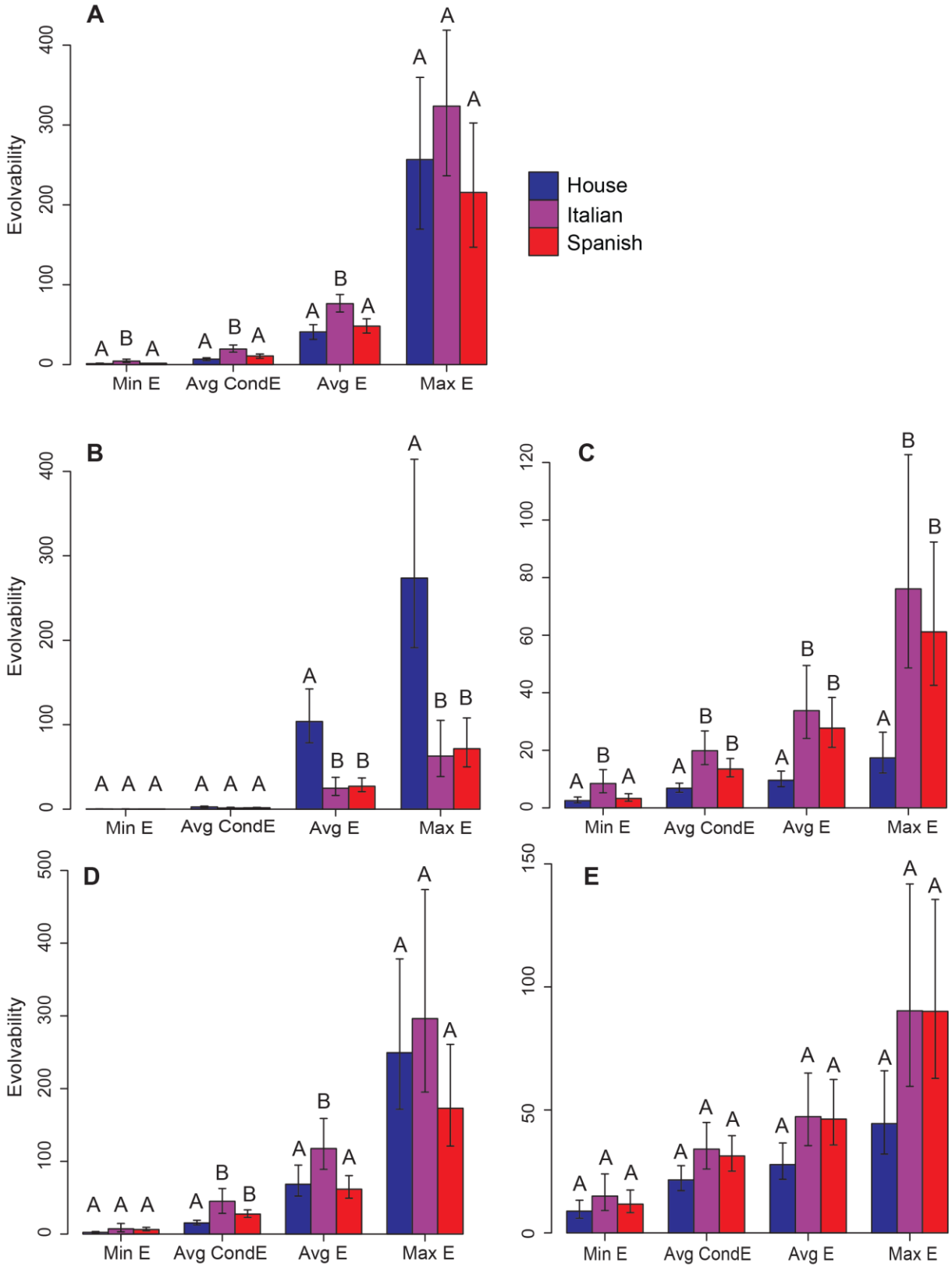


Figure 11. The estimated potential for each species to respond to selection. Shown in: all plumage traits except crown (A), crown (B), back (C), rump (D) and cheek color (E). Each plot shows the median of minimum evolvability (Min E), the median of average conditional evolvability (Avg Cond E, $c_{\bar{y}}$), the median of average evolvability (Avg E, $e_{\bar{y}}$) and the median of maximum evolvability (Max E) for the respective species. Whiskers represent the 2.5 and 97.5% quantiles. Letters indicate the level of significance between groups.

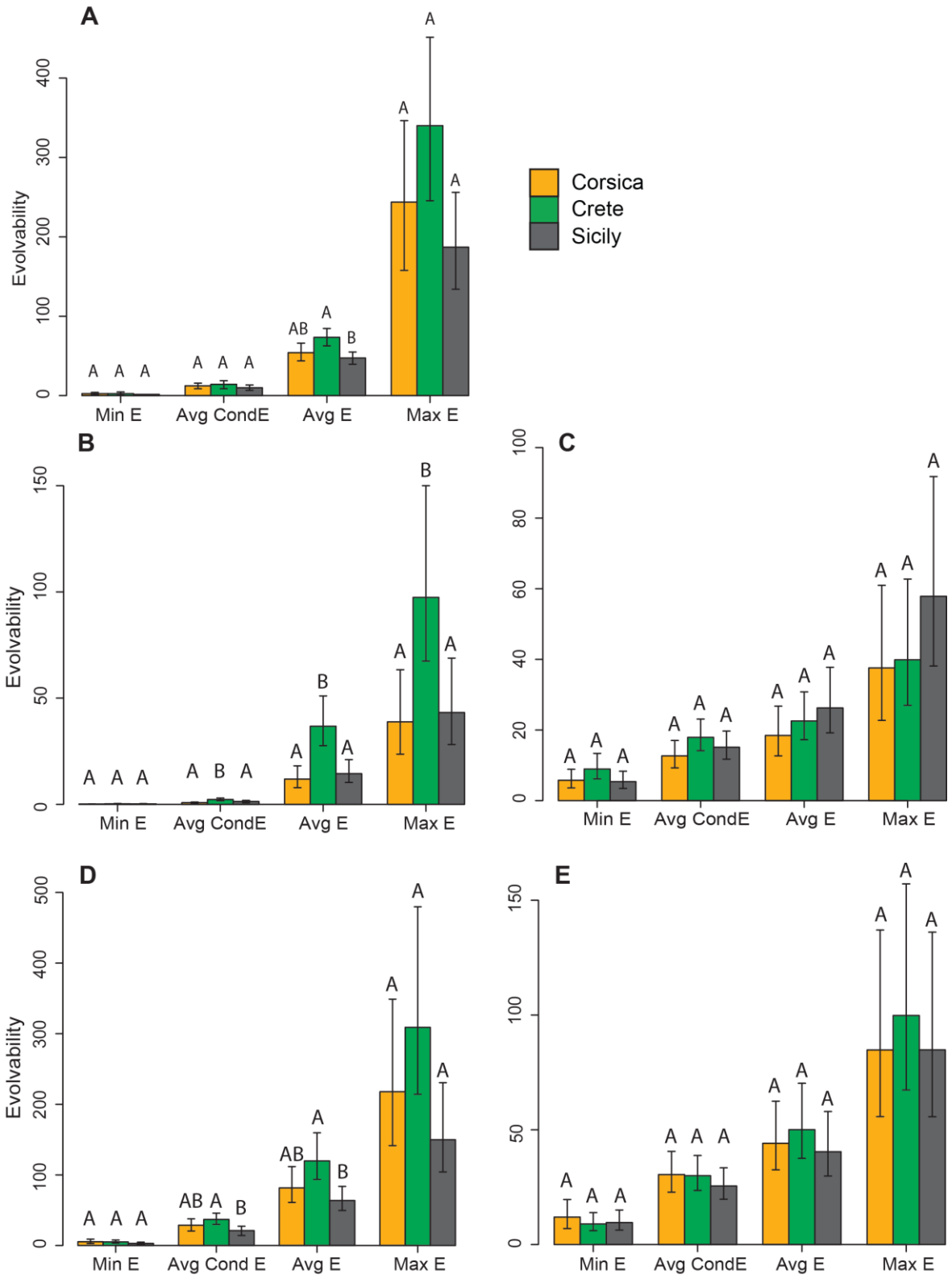


Figure 12. The estimated potential for island populations of Italian sparrows to respond to selection. Shown in: all plumage traits except crown (A), crown (B), back (C), rump (D) and cheek color (E). Each plot shows the median of minimum evolvability (Min E), the median of average conditional evolvability (Avg Cond E, $c_{\bar{y}}$), the median of average evolvability (Avg E, $e_{\bar{y}}$) and the median of maximum evolvability (Max E) for the respective islands. Whiskers represent the 2.5 and 97.5% quantiles. Letters indicate the level of significance between groups.

Average autonomy (*a*) is a measure of the average proportion of trait variation uncorrelated with other traits across all axes of trait variation, and hence on average how independently a trait can evolve. Average autonomy varied between species (Fig. 13), but there were no significant differences between species or between islands for any individual trait or all combined (Fig. 13, Table S3-S7). However, there were some consistent patterns. For combined plumage traits, Italian sparrows had the highest average autonomy, while the Italian sparrow was intermediate between the two parental species in each trait separately (Fig. 13A). For back- and cheek plumage house sparrows had the highest autonomy, while Spanish sparrows had the highest autonomy for rump plumage. Crown autonomy was severely reduced in all three species.

As in the species comparison, the island populations of the Italian sparrow did not differ significantly in their average autonomy in each trait (Fig. 13B; Table S3-S7). Corsica had the highest autonomy for rump color, and for cheek color. Crete had the lowest autonomy in all traits except back color, where it had the highest autonomy.

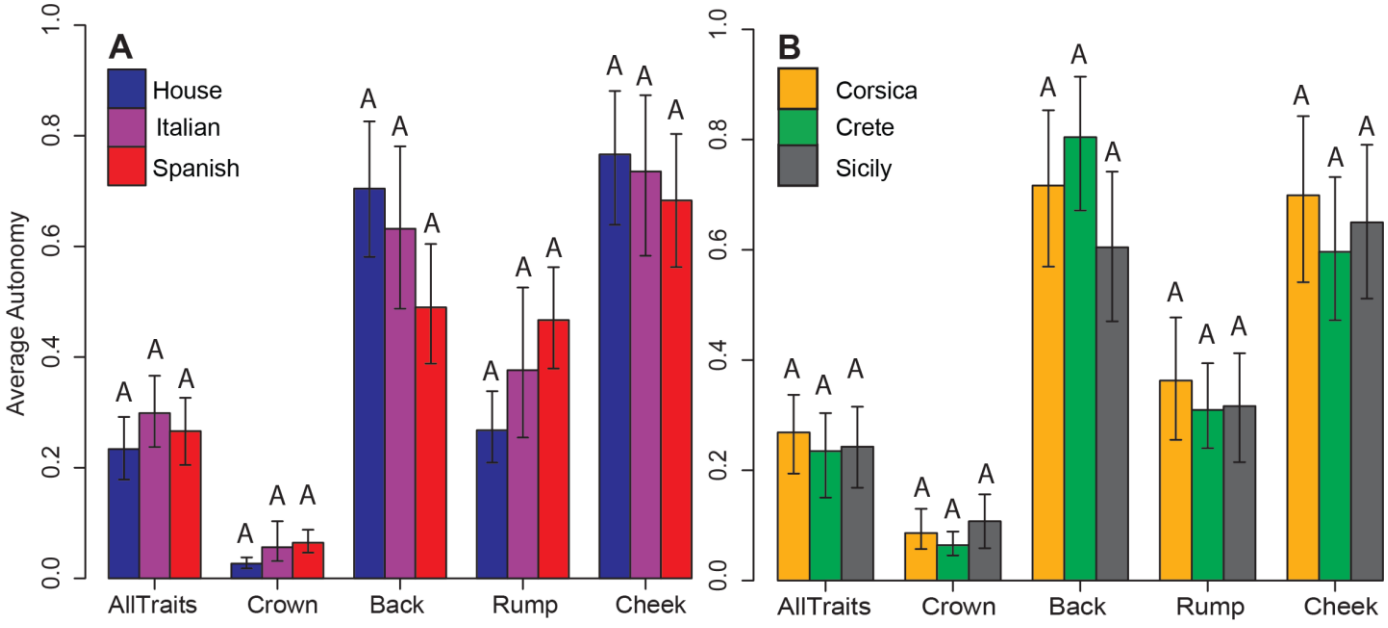


Figure 13. The average amount of variation able to evolve independently from correlated traits between species and between islands. The median of average autonomy is shown for species (A) and Italian sparrow island populations (B) for each respective trait. 'AllTraits' includes back, rump and cheek plumage color. Whiskers represent 2.5% and 97.5% quantiles. Letters indicate levels of significance between groups.

The extent of constraints along the parental axis of differentiation

The results of the evolvability ($e(\beta)$) and conditional evolvability ($c(\beta)$) measures along the parental axis of differentiation (the discriminant axis) were compared with the average, minimum and maximum evolvability described above. High evolutionary potential along this axis would be revealed by an $e\beta$ or $c\beta$ value greater than the average. The results showed that all three sparrow species were less capable of evolving along this axis than they were on average. Thus, Italian sparrows did not have excessive evolutionary potential in this direction, but rather were equally constrained as the parent species (Fig. 14). Back plumage color was the only trait where the Italian sparrow had higher both evolvability and conditional evolvability along the parental axis compared to average evolvability, a value that also was significantly higher than those of the parental species (House 2.5% quantile of median $e(\beta)$ = 7.64, 97.5% = 16.85; Italian 2.5% quantile median $e(\beta)$ = 33.26, 97.5% = 85.89; Spanish 2.5% quantile median $e(\beta)$ = 6.45, 97.5% = 14.00; Fig 14C; Table S5). Spanish sparrows appeared less evolvable along the parental axis of differentiation than average values in each individual trait, but appeared higher for combined traits (back, rump and cheek) (Fig. 14). House sparrows, on the other hand, had a lower than average conditional evolvability in back, rump and cheek plumage whereas they had a higher than average evolvability in back plumage, as well as a higher than average evolvability and conditional evolvability in crown plumage color (Fig. 14).

Comparing islands, Italian sparrows on all three islands were estimated to be less capable of responding to selection along the discriminant axis than on the average across all potential selection gradients (Fig. 15). For back plumage, Sicily had the highest conditional evolvability and evolvability along the parental axis (Fig. 15C). For rump color, Crete was most evolvable along this axis, and for cheek color, Corsica was the most evolvable (Fig. 15). Corsica appeared to have the highest evolvability, and Sicily the lowest evolvability, along the parental axis when considering back, rump and cheek plumage combined (Fig. 15A). Corsica's pattern of capability of response to selection is not significantly different from that of either parent species for back color (Fig. 15; Table S5). In contrast, the amount of evolvability along the parental axis for Crete and Sicily is significantly higher than those of house and Spanish sparrows for back plumage (Fig. 15; Table S5).

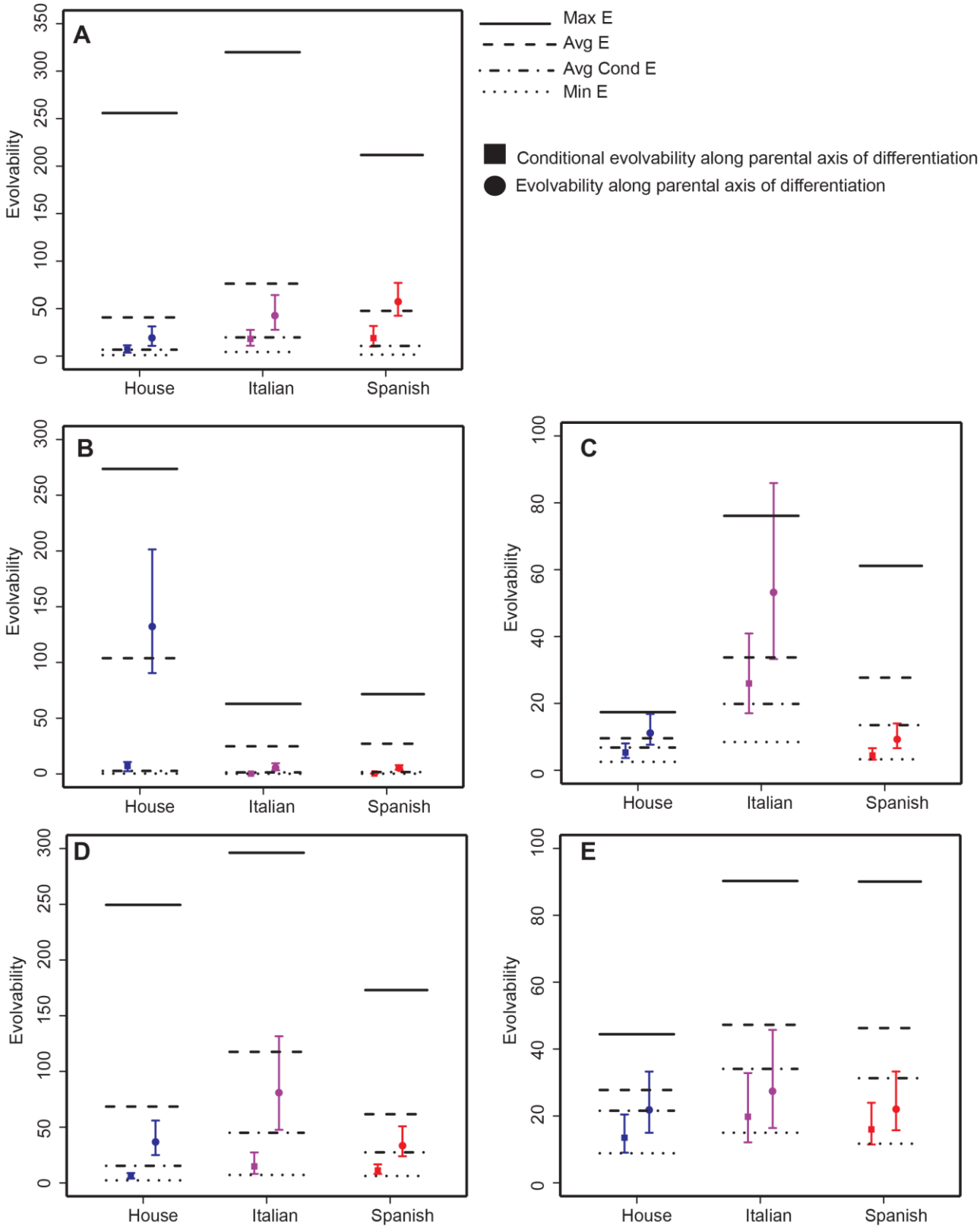


Figure 14. Evolvability along the parental axis of differentiation in comparison to species average evolvability. Shown for: all traits except crown (A), crown (B), back (C), rump (D) and cheek (E) plumage color. The square denotes the median conditional evolvability and the filled circle represents the median evolvability along parental axis of differentiation for each respective species per trait. Horizontal lines indicate population means for each respective evolvability statistic. Whiskers represent 2.5% to 97.5% quantiles.

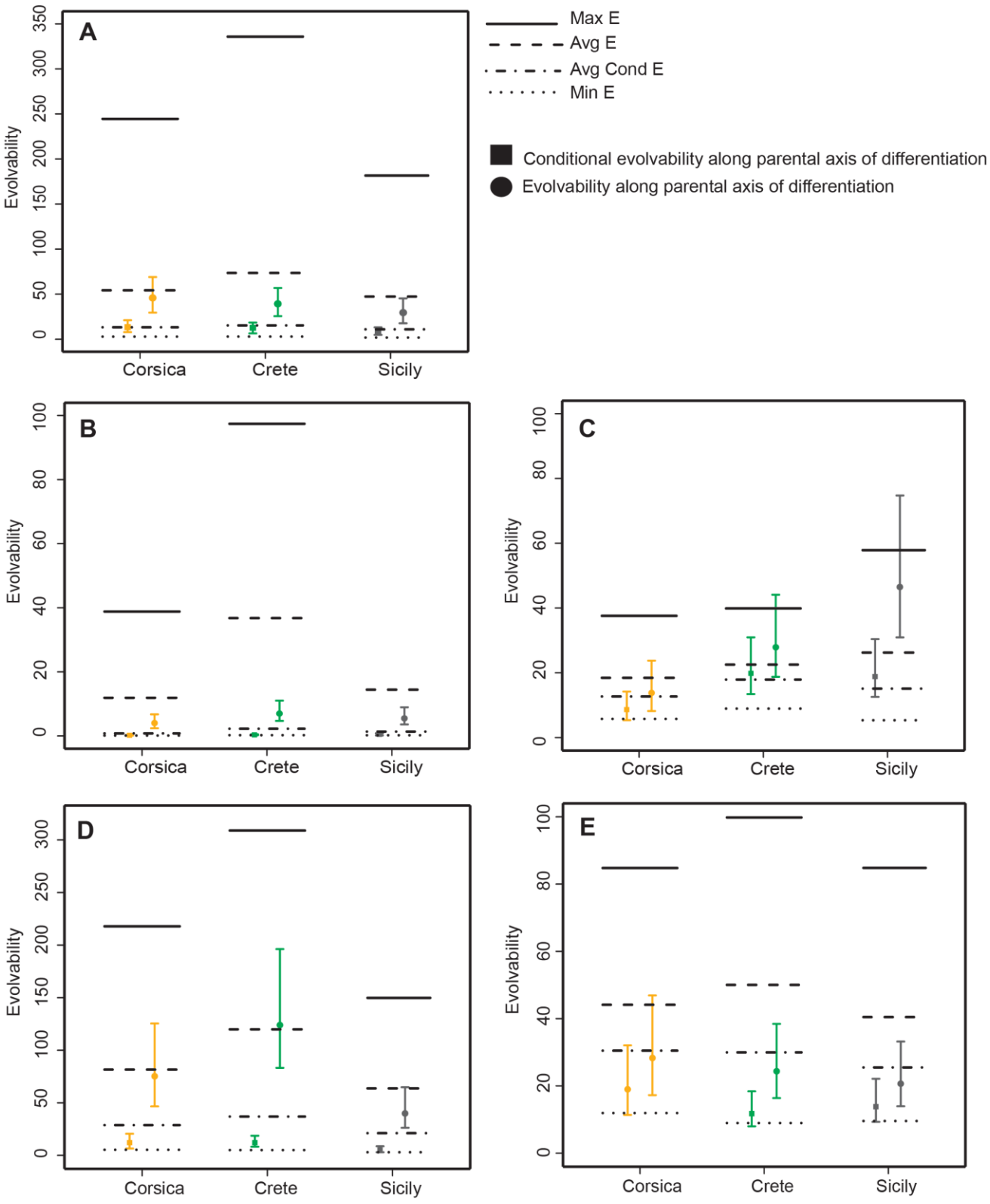


Figure 15. Island evolvability along the parental axis of differentiation in comparison to island average evolvability. Shown for: all traits except crown (A), crown (B), back(C), rump (D) and cheek (E) plumage color. The square denotes the median conditional evolvability and the filled circle represents the median evolvability along parental axis of differentiation for each respective island per trait. Horizontal lines indicate population means for each respective evolvability statistic. Whiskers represent 2.5% to 97.5% quantiles.

The similarity of species' and islands' response to selection

Random skewers analysis revealed that Italian sparrow responses to selection would be significantly correlated to those of Spanish sparrows, but not those of house sparrows (Fig. 16A; Appendix II, Table S9; Table S10). Spanish sparrows and all individual islands for Italian sparrows would show significantly correlated responses to selection, but none were significantly correlated with house sparrow responses (Fig. 16B; Table S9; Table S10). Corsica and Crete were very similar to each other as well as to Spanish sparrows, and their expected responses to selection fell on the axis of differentiation between the parent species. Sicilian Italian sparrows, however, showed a somewhat transgressive pattern of expected responses. Across all analyses, Sicilian sparrows appeared somewhat different in their trait variation from the other two islands.

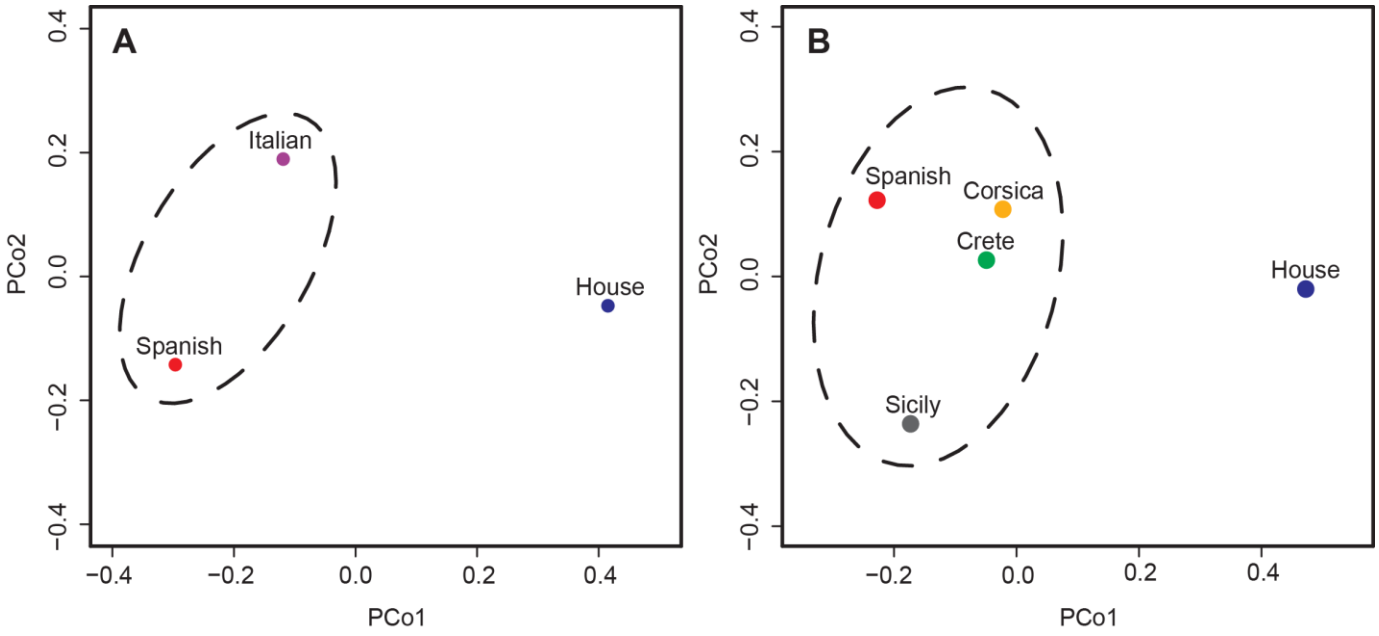


Figure 16. Principle coordinates plot showing how similar the plumage traits would respond to selection, comparing species (A) and islands and parentals (B). Ellipses indicate groups that are significantly correlated in their expected response to selection based on random skewers analysis.

Discussion

The amount of variation in color between island populations of Italian sparrows, and the varying degree of similarity between islands and the different parent species, indicates that the Italian sparrow is not constrained to form a specific phenotypic combination and has a high potential for further diversification among – though not necessarily within – isolated islands. Furthermore, alongside their mosaicism and intermediacy in plumage color patterns, some Italian sparrows have transgressive plumage for two traits: back and rump. Hybridization between a specific pair of parental species can thus be a potential source for not only one, but many new phenotypic forms of secondary sexual plumage combinations. Furthermore, the Italian sparrow has, on average, more plumage diversity and hence is more capable of responding to selection pressures compared to its parent species, which implies that hybridization can increase the ability for a species to adapt and evolve. However, for all traits except back, any increase that might have occurred in hybrid evolutionary potential along the primary axis of color differentiation between the parents has been lost. Meanwhile, hybridization has not caused a systematic increase or decrease in within-trait evolutionary constraints via changes in autonomy, but there is some suggestion that the different plumage traits are more autonomous from each other in the Italian sparrow than in the parents. Finally, while Italian sparrows show different overall patterns of expected responses to selection compared to house sparrows, all island populations would be expected to evolve in similar ways to their other parent, the Spanish sparrow.

Evolutionary potential in hybrids is likely to be strongly affected by three factors: the magnitude and pattern of divergence between the parent species, the selection pressures acting on the traits in hybrid populations, and the time those hybrid populations have had to evolve. Traits that diverge between the parental species primarily by directional selection should lead to hybrids with non-transgressive trait values stretched along the axis of parental divergence (Rieseberg et al., 1999, Bailey et al., 2013). Genetic divergence in traits under stabilizing selection or drift is less likely to be correlated with phenotypic divergence, and can thus cause transgression in hybrids. However, this requires strong genetic differentiation. Traits that remain polymorphic, with similar underlying allele frequencies in both parents, will show little or no increased evolvability or transgression in hybrids, at least through additive effects. These factors may help explain the patterns of differentiation and evolvability in the male plumage of Italian sparrows.

Italian sparrows are thought to have first formed some 10 000 to 4 000 years ago (Hermansen et al., 2011, Sætre et al., 2012). Although it is known that the island populations differ in their current genomic composition (Runemark e al. in prep), it is not yet known whether all Italian sparrows have a single origin, or whether multiple independent hybridization events occurred. Nor is it known whether there is any variation among locations in the time since hybridization and hybrid speciation. However, if we assume that Italian sparrows have had some thousands of generations to evolve, as seems likely, this may be enough time for selection to act and to stabilize the hybrid genome (Buerkle and Rieseberg, 2008). The lack of plumage variation along the axis of parental differentiation in the hybrids – with the exception of back plumage - is therefore likely to be the result of evolution since the hybrid species formed. The fact that variation along this axis has been mostly lost, while the hybrids maintain high overall plumage diversity relative to their parents, suggests that strong selection has been acting primarily along this axis. Earlier studies suggested that postzygotic incompatibilities have undergone strong directional selection in mainland Italian populations, leading to a mosaic pattern of purging of alleles from one parent and near-fixation of the alternative allele from the other parent (Trier et al., 2014). Similar purging may have occurred in sexual signals, through genetic correlations with incompatibilities, ecological selection, or sexual selection via female choice or male-male interactions. High evolvability along this axis in early generation hybrids may have led this to represent a genetic 'line of least resistance' (Schluter, 1996), with exceptionally high initial evolvability interacting with strong selection to subsequently remove much of the variation and push the hybrid phenotype towards one parent or the other, depending on the trait in question.

Selection pressures may differ between sparrow plumage traits, as has been shown in other multivariate sexual signaling systems (Bentsen et al., 2006), and this may explain some of the differences in hybrid evolutionary potential described here. The Italian sparrow has a mosaic plumage pattern, being partially transgressive in back color but more house-like on Corsica and Crete and more Spanish-like on Sicily, and intermediate, but more similar to Spanish sparrows in rump color (with some transgression in Sicily), Spanish-like in cheek color with no transgression, and finally, almost fixed for Spanish sparrow crown color. I now discuss some possible explanations for the differences between traits in hybrid evolutionary potential. While this discussion is somewhat speculative, it is based on predictions from population genetic theory (Rieseberg et al., 1999; Bailey et al., 2013).

Crown and cheek are more fixed towards one of the parental species compared to some of the other traits, and have very little variation along the parental axis of differentiation in all species and islands (with the exception of crown color in house sparrows). While cheek color has a lot of individual-level variation in all species, neither of these traits is transgressive in Italian sparrows. One potential explanation for these observations is that crown and cheek may be under strong directional sexual selection. Sexual selection on plumage characters is well-known (e.g., Greene et al., 2000, Pryke and Griffith, 2006), and crown color has previously been shown to be an important trait for sexual selection in the Alpine hybrid zone between Italian and house sparrows (Bailey et al., 2015). Male house sparrows chirp to attract females to their territory, and if a female approaches, the male will chirp rapidly, hold out and droop his wings and tip his head back, while raising and spreading his tail feathers (Summers-Smith, 1988, pg. 145-146). Italian and Spanish sparrows engage in similar displays (G-P Sætre personal observations). During this act, back and rump plumage may be less visible, while crown and cheek are more conspicuous. It is possible that the Italian sparrow females have inherited a preference for brown crowns and white cheeks from Spanish sparrows, leading to purging of both gray crowns and house sparrow-like cheek plumage through strong sexual selection in the hybrid species. Another potential explanation for the observed pattern in crown color is the effect of dominance; brown crown color could be completely dominant over gray. This is likely, as F_1 hybrids were shown to have Spanish sparrow chestnut crowns (Macke, 1965). However, a cline analysis of the Alpine hybrid zone revealed that most of the discovered SNPs between house and Italian sparrows were concentrated in the area where gray crowns were common, thus disproving this theory (Bailey et al., 2015). The presence of high levels of variation in other aspects of cheek plumage in all species, and the apparent absence of transgression in this trait, suggests that only certain aspects of cheek plumage are under sexual selection, with the rest representing neutral variation and remaining polymorphic in all species. Cheek color could also be condition-dependent, rather than heritable, causing high variability. However, despite significant differentiation in cheek color across the Alpine house-Italian sparrow hybrid zone, there was no strong evidence that cheek was contributing to sexual isolation in this region (Bailey et al., 2015). This may reflect a lack of sexual selection acting on this trait, or it may partially reflect the lower data quality in this previous study, which did not employ light standardization techniques or a color checker. For crown color, the high variation in house sparrows along the axis of differentiation suggests that

sexual selection may be weaker on this trait in the house sparrow than Italian and Spanish sparrows.

Rump color has relatively low within-trait autonomy for all three species, and as such, may be constrained to evolve in a few specific directions. Similar to cheek color, rump has little variation along the axis of parental differentiation in all three species, suggesting strong selection along this axis. However, it differs from cheek in being partially transgressive in Italian sparrows, particularly on Sicily, having more between-island variation (Sicily being differentiated from Corsica and Crete), a greater increase in average evolvability in the hybrid species, and in being relatively intermediate in hybrid phenotype rather than matching one or other parent species. These patterns point towards greater parental genetic differentiation and lower parental polymorphism than cheek color along axes that do not contribute to species differentiation. Overall, this suggests that rump variation is under stabilizing selection in the parents, rather than evolving by drift. Low variability but intermediate trait values in hybrids along the parental axis of differentiation further suggest that the mechanisms of selection may differ from those for crown and cheek, possibly being more multivariate or polygenic, with elements inherited from both parents.

Unlike rump color, back color has high within-trait autonomy for all three species, and is moderately evolvable for Italian and Spanish sparrows. In addition, particularly for Italian sparrows, back is more evolvable than average along the parental differentiation axis, both overall and for Crete and Sicily. It is also the trait showing most transgression, particularly in Sicily. This suggests that back plumage was under stabilizing selection in both parent species, and that the initial high levels of variation in the hybrid have not subsequently been removed by selection. In this scenario, the Italian sparrow back plumage could be under weak stabilizing or directional selection. Taking into consideration that Italian sparrow back plumage displays the most transgression of the plumage traits this means that back color follows the prediction that hybrids established by parent species under stabilizing selection are more likely to exhibit transgression.

There are thus some indications that rump and back plumage are under selection. Potentially, rump and back color may still be under sexual selection despite being less visible, as all traits analyzed in this study are exclusive to male birds. The observed differences between back and rump color and the other traits could also be due to natural selection pressures. One factor that could possibly influence plumage color is photo-protection. The pigments responsible for

sparrow plumage color are divided in two types; the black-to-brown eumelanin and the yellow-to-red pheomelanin (Prota et al., 1995), and of the two, eumelanin has been shown to be more resistant to the sun. Rump plumage, while concealed when the bird is perched, is exposed during flight, and hence, there could have been natural selection for eumelanin based rump color. Plumage color traits can also be naturally selected to mediate crypsis. Dull colors might provide camouflage against common predators, such as sparrowhawks and domestic cats (Summers-Smith, 1988). The strength of camouflage might depend upon all plumage traits as a whole, and therefore, selection pressure may have developed genetic correlations between sets of plumage traits.

The evolutionary potential of hybrid species

The Italian sparrow is, on average, more evolvable than the parent species in all plumage traits except crown color, and exhibits some transgression in rump and back color (Fig. 4; Fig. 12; Fig.14). This is consistent with hybridization being a potential source for diversity (Rieseberg et al., 1999, Dittrich-Reed and Fitzpatrick, 2013). The hybrid species has access to both sets of genes during the speciation process, which may increase its variability and thereby its potential to respond to selection, especially if there are numerous loci fixed for different alleles in each parent. Interestingly, the Italian sparrow is more autonomous than its parent species when considering all its plumage traits simultaneously, but it is intermediate between the two parent taxa for each trait independently. This could mean that the different plumage traits have become partially genetically decoupled in the Italian sparrow. The mechanisms are unknown, but should this be a common phenomenon, hybrid species may have increased potential for new diversity and mosaicism, and a greater capability to evolve along random trajectories compared to their parent species.

Increased evolvability and autonomy in hybrids relative to parent species has previously been observed in F_2 hybrids between African chiclids (Parsons et al., 2011), and a number of studies have shown hybrid species with novel phenotypes, which is a sign of increased potential for evolution (e.g., Rieseberg et al., 2003, Gompert et al., 2006, Fitzpatrick and Shaffer, 2007). However, the reduced variability along the axis of parental differentiation in Italian sparrows suggests that this increase may often be a temporary phenomenon,

particularly in traits such as sexual signals that are likely to be under strong selection, and therefore only present in the early stages of hybrid evolution.

Selection may also affect the hybrid species differently from the parent lineages. Italian sparrow plumage traits would respond differently than the parent species to selection due to their differing P-matrices. This could drive the hybrid to evolve along its own trajectory. For instance, Italian sparrow rump color has little variation along the parental axis of differentiation and is more integrated and less variable than cheek and back color, yet still displays some transgressive values. This could mean that similar selection pressures on the three sparrow species has influenced the Italian sparrow rump trait to evolve in a different direction than house and Spanish sparrows.

The presence of novelty does not necessarily mean that a population has high variability, nor does it follow that novelty always increases the potential for a population to respond to selection. Studies on hybrid sunflowers report lower genetic variation in the hybrid species than in the parent taxa (Edelist et al., 2006, Gross et al., 2007). These sunflowers are separated from the parent species due to adaption to extreme habitats, and strong selection pressure may have reduced the amount of available variation. The Italian sparrow apparently shares the same niche as the house sparrow. Thus, transgression might not have been as crucial for the Italian sparrow in order to establish itself as a species, largely in allopatry, in Italy and Mediterranean islands. However, reproductive isolation had to develop in sympatry with its parent species, and sexual selection and/or selection for assortative mating may have been important for establishing reproductive isolation. This may have affected its potential for diversity if female mate preferences have constrained the Italian sparrow to evolve along the parental axis of differentiation.

Island contingencies; how constrained is hybridization to follow one trajectory?

Italian sparrows vary in color between individuals, and the three island populations, Corsica, Crete and Sicily, differ from each other with respect to plumage color. Since the Italian sparrow varies in coloration, it is clear that more than one combination of hybrid phenotype can evolve.

Variation between individuals and populations does, however, generally make up higher proportions of the overall variation than that of the island level, implying that the three islands have undergone parallel evolution to some extent. This suggests that the phenotype is not strongly affected by the island-specific genetic composition (Runemark et al. in prep). Nonetheless, the amount of transgression differed between islands, and the three populations varied in the degree of resemblance to each parent species for back, rump and cheek color. One of the most striking patterns of my analysis is the extent of divergence between Sicily and the other two islands. Sicilian sparrows appear to resemble Spanish sparrows more than Corsica and Crete, and exhibit more transgression. Previously, Summers-Smith (1988) has qualitatively described the similarity to Spanish sparrows on Sicily, and similar phenotypes also exist on the adjacent mainland in Calabria, south-west Italy. Sicily also has the highest proportion of Spanish sparrow ancestry of the three island populations (Runemark in prep.), so this result could partly reflect the greater contribution of Spanish sparrow alleles to the genome compared the other two island populations. In fact, Summers-Smith (1988) suggested that Calabrian and Sicilian Italian sparrows may be backcrosses between Italian and Spanish sparrows based on phenotypic considerations, but genetic analysis suggest the presence of Italian-specific gene combinations of important genes through the entire distribution of the species (Trier et al., 2014).

Local selection pressure may also have led the populations to diverge in traits that are not as constrained by genetic correlations or incompatibilities, and simultaneously maintain parallel evolution in traits under strong parallel selection or with strong genetic correlations to incompatibilities. Separate hybrid lineages have been reported in other study systems (e.g., Wang et al., 2001, Gross et al., 2007, Nice et al., 2013). Homoploid hybrid butterfly lineages from the crossings of *Lycaeides melissa and L. anna* had wing patterns that varied between populations; one population exhibited transgressive wing patterns, whereas three of the populations were not significantly different from one of the parent species (Nice et al., 2013). Similarly, none of the Italian sparrow populations differ significantly from Spanish sparrows in cheek and crown color (Table 2). As previously discussed, selection pressure could have influenced both these hybrid species to evolve towards one of the parent species, as it is often the direction of least resistance. Unlike the butterflies, none of the sparrow populations have diverged to the point where all individuals are transgressive for any trait. The amount of time selection has had to act may have affected the extent of divergence. Weak selection pressure will cause populations to diverge more slowly, especially if the adaptation requires the

population to overcome a set of constraints. The alpine butterflies are estimated to have established as a hybrid species 442 579 years ago (Gompert et al., 2006), while the Italian sparrow is thought to have originated 10 000 years ago or less (Hermansen et al., 2011; Sætre et al. 2012), a relatively short amount of time in terms of evolution. As such, the Italian sparrow has had less time to undergo novel mutation and to evolve novel phenotypes, and could therefore have reduced amounts of transgression. Hybrids between cichlid species have been shown to be more transgressive when the parents are more divergent (Stelkens and Seehausen, 2009), and transgressive values were also seen in laboratory crosses of cichlids (Stelkens et al., 2009).This suggests that the amount of transgression is not necessarily affected by time. Most likely, transgression can also depend on the genetic processes that underlie a trait, such as complementary gene action and epistasis.

The genetic processes underlying the phenotypic traits in the parental species has a major effect on the potential for hybridization to develop novel phenotypes and phenotypic combinations (Bailey et al., 2013). Identifying candidate genes coding for plumage color in the Italian sparrow, and inferring how they function, would complement this study and give us further insight into how hybridization can contribute to new variation and affect the potential for evolution. This is an exciting avenue for future research. Furthermore, it would be interesting to separate and categorize plumage colors into the corresponding pigments, eumelanin and pheomelanin, to connect the genetic background with the potential for constraints within and between metabolic pathways of each pigment.

In this study, a phenotypic P-matrix was used to study the evolutionary potential of a hybrid species, when commonly researchers use an additive genetic variance matrix, or G-matrix. Although the phenotypic correlations might not accurately reflect the genetic correlations, increasing evidence shows that the differences between phenotypic correlations and genetic correlations are minor enough for a P-matrix to roughly represent a G-matrix (tested in Cheverud, 1988, Roff, 1995, Steppan, 1997, Waitt and Levin, 1998). Further, no study thus far has analyzed the heritability of plumage traits in the sparrow taxa. The level of heritability of plumage traits may explain the type of selection acting on that particular trait; melanin based plumage traits are predicted to be highly heritable, as has been shown in other bird taxa (e.g., Hill and Brawner, 1998), while carotenoid based colors are more condition-dependent (e.g., Hill, 1990), and plumage traits affected by ecological factors are expected to be partially heritable and partially condition-dependent (e.g., Slagsvold and Lifjeld, 1992). Sparrows do

not have any carotenoid-based colors, which may reduce the likelihood that the traits analyzed here are condition-dependent.

This study represents the first objective quantification of male plumage color differences between Spanish sparrows and either house or Italian sparrows, as well as an expanded and more rigorous examination of house-Italian sparrow differentiation than previously carried out (Bailey et al., 2015). I have shown that hybridization is a potential source for new variation, as the Italian sparrow has higher variability than the parent species and displays novel phenotypes. Furthermore, hybridization between the same parent species can form more than one phenotypic combination, although homogeneity of selection pressures and degree of parallelism may differ between traits. Moreover, hybrid species can be more variable and more evolvable than parent species, as seems to be the case for the Italian sparrow, but can rapidly lose variation along the axis of parental divergence. The role of hybridization in contributing novel variation for selection to act upon is an exciting field for future research, and further research on sexual signaling systems, including examination of mate choice, can help further our understanding of how sexual selection contributes to homoploid hybrid speciation.

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Appendix I

Supplementary Figures

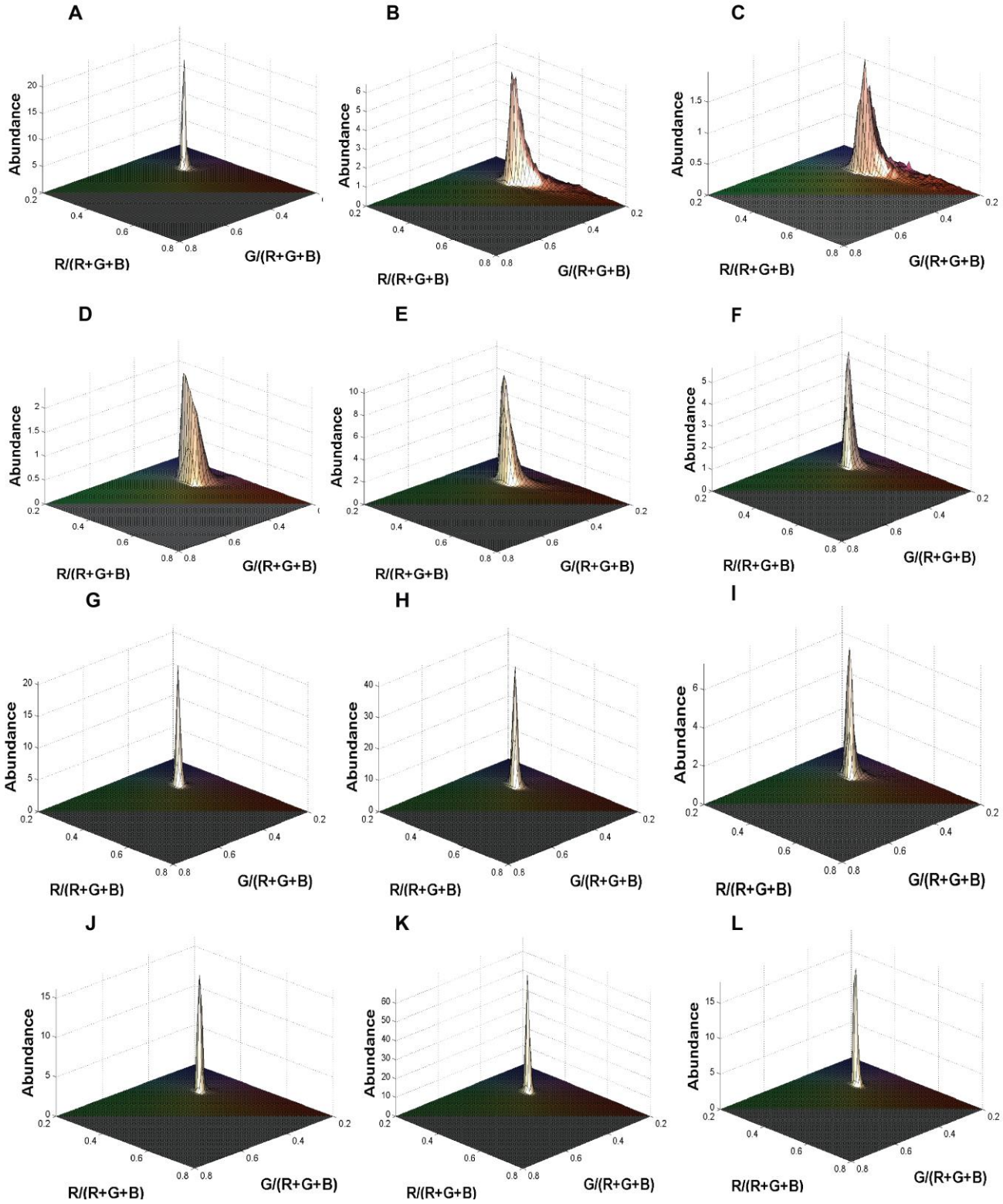

Figure S1 The chromaticity of house, Italian and Spanish sparrow plumage per trait. Shown for crown (A-C), back (D-F), rump (G-I) and cheek (J-L), where the species are represented per column; house sparrows (A,D,G,J), Italian sparrows (B,E,H,K) and Spanish sparrows (C,F,I,L).

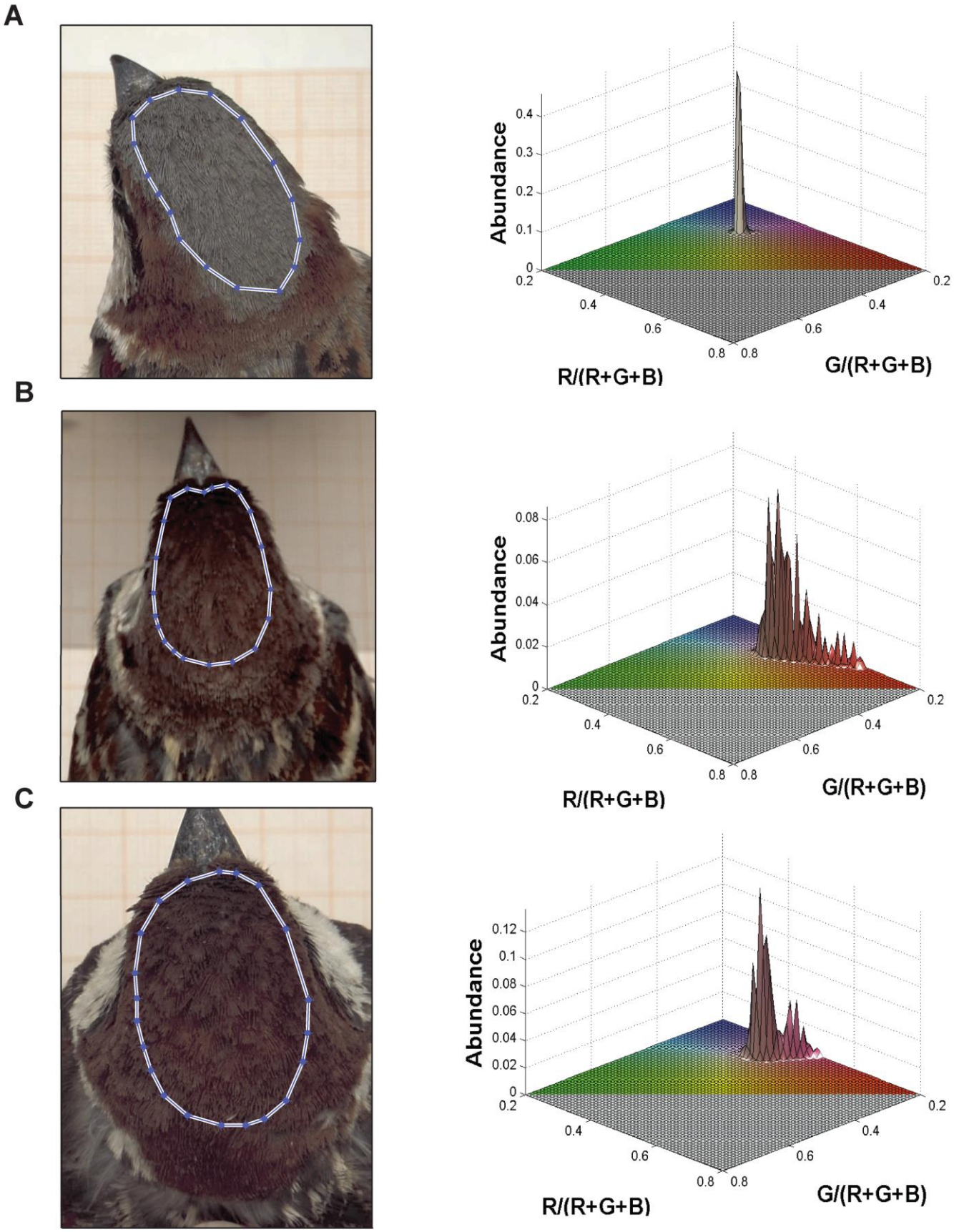

Figure S2. Examples of crown plumage color for each sparrow species. Chromaticity histograms represent the color of selected area in the photo to the left, for house sparrows (A), Italian sparrows (B) and Spanish sparrows (C).

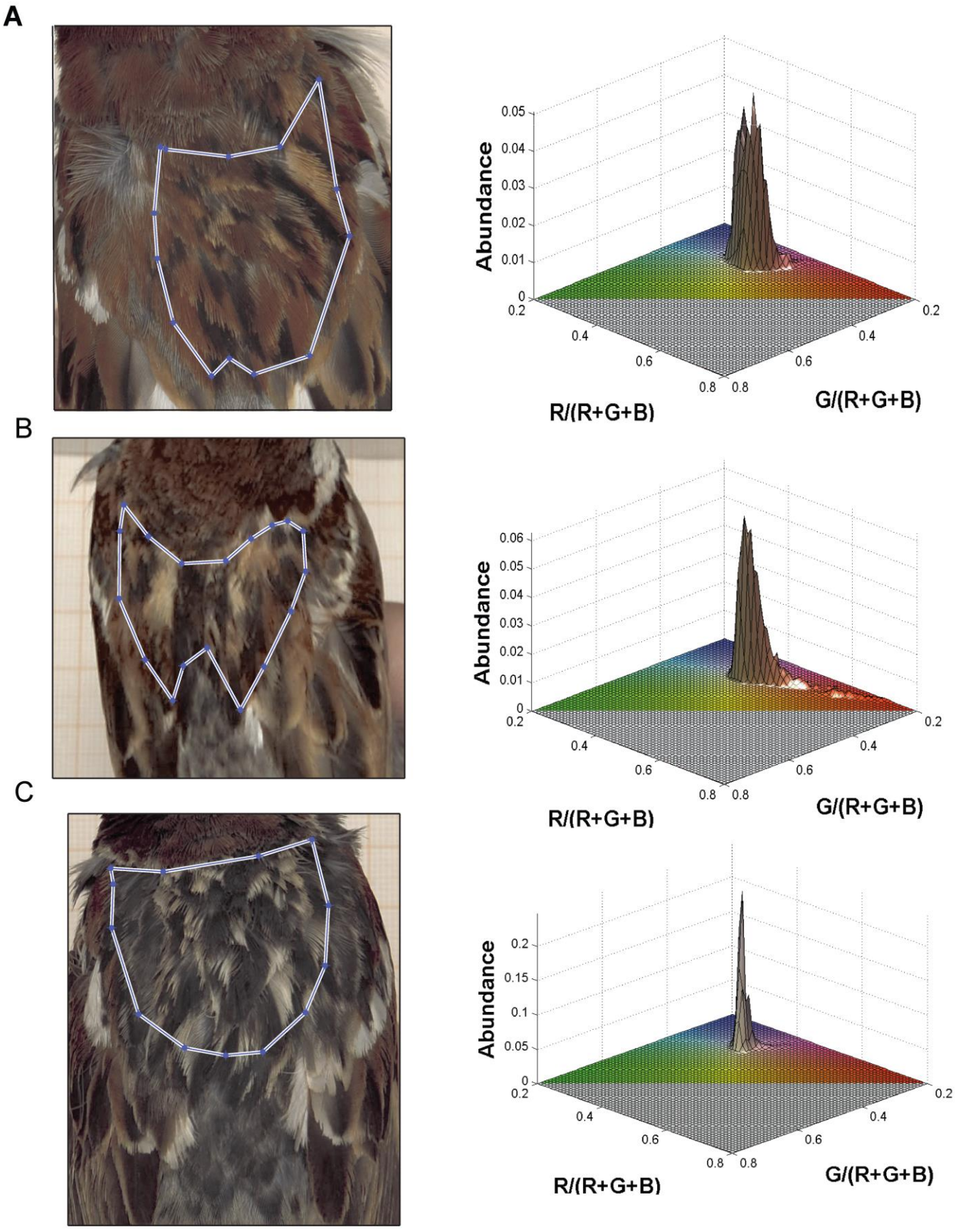

Figure S3. Examples of back plumage color for each sparrow species. Chromaticity histograms represent the color of selected area in the photo to the left, for house sparrows (A), Italian sparrows (B) and Spanish sparrows (C).

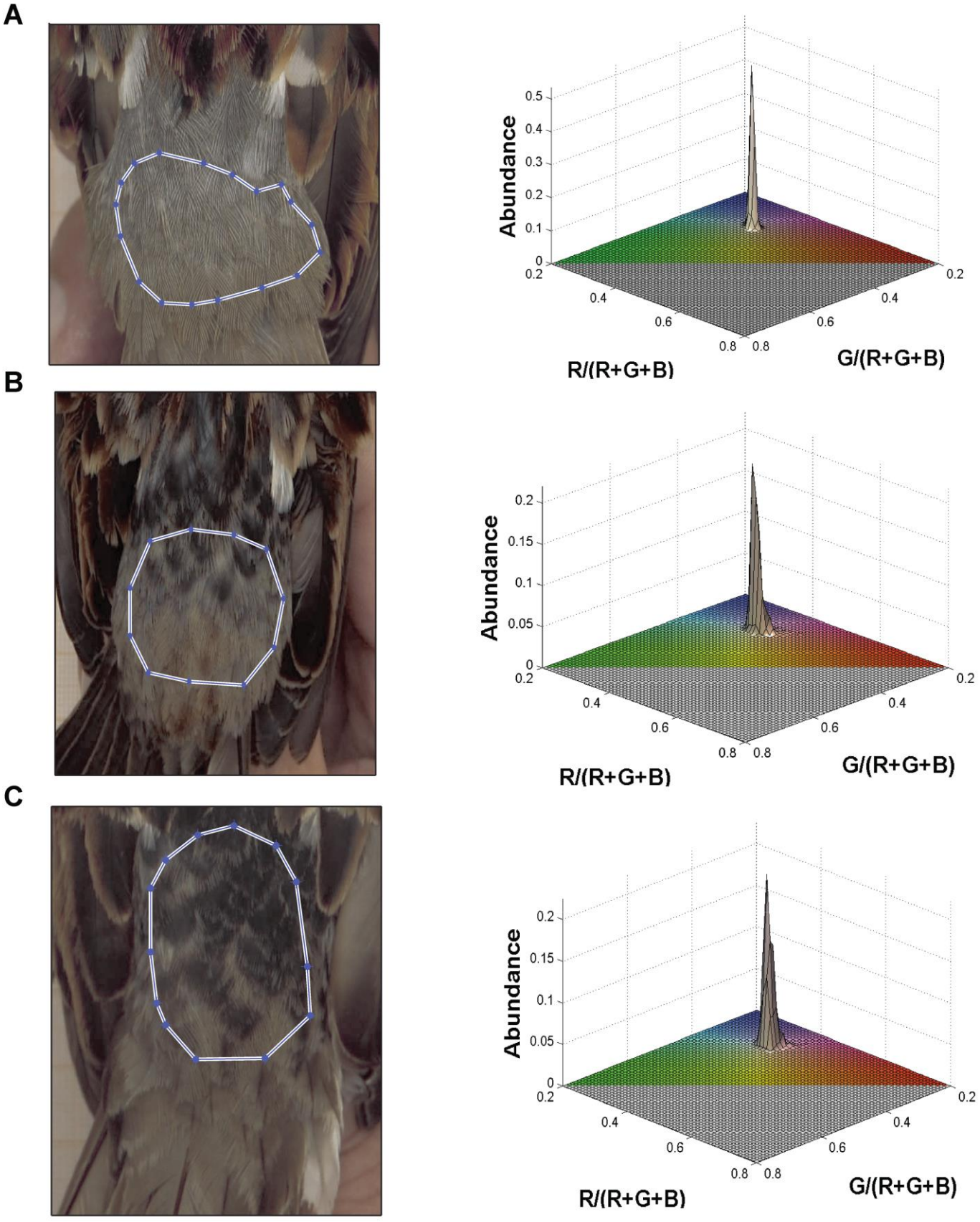

Figure S4. Examples of rump plumage color for each sparrow species. Chromaticity histograms represent the color of selected area in the photo to the left, for house sparrows (A), Italian sparrows (B) and Spanish sparrows (C).

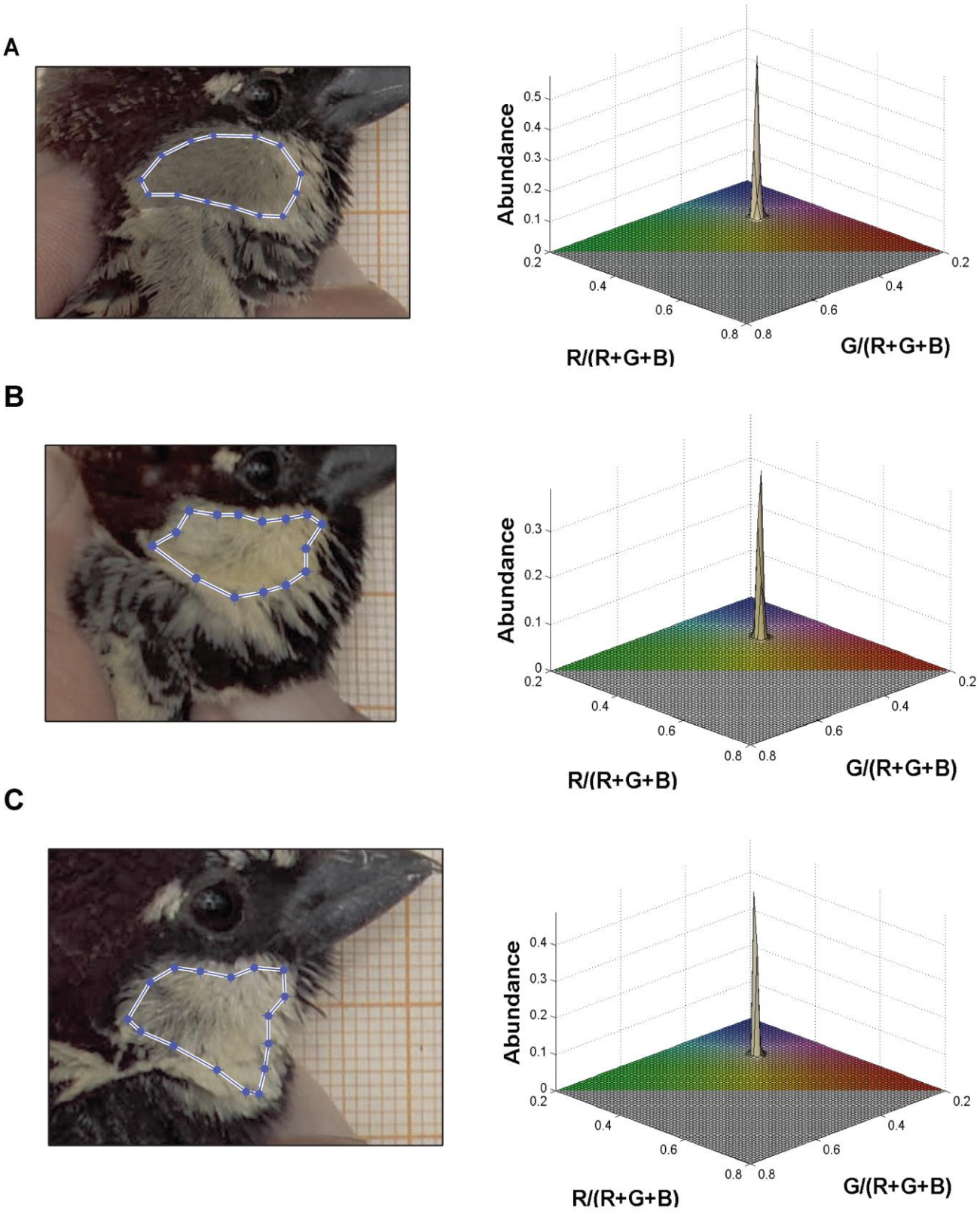

Figure S5. Examples of cheek plumage color for each sparrow species. Chromaticity histograms represent the color of selected area in the photo to the left, for house sparrows (A), Italian sparrows (B) and Spanish sparrows (C).

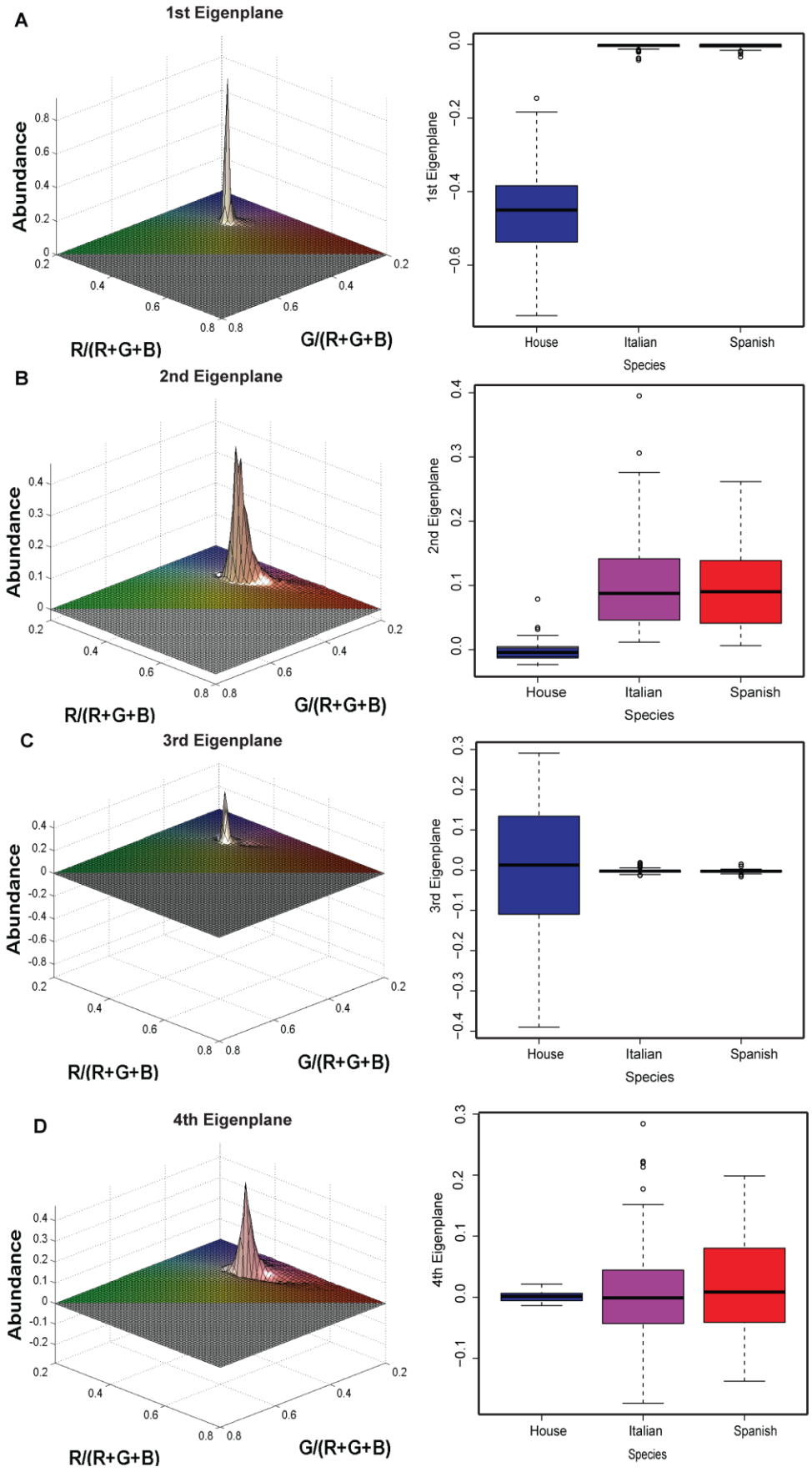

Figure S6. Crown plumage chromaticity and the amount of variation between species for each eigenplane; shown in the 1st eigenplane (A), 2nd eigenplane (B), 3rd eigenplane (C) and 4th eigenplane (D).

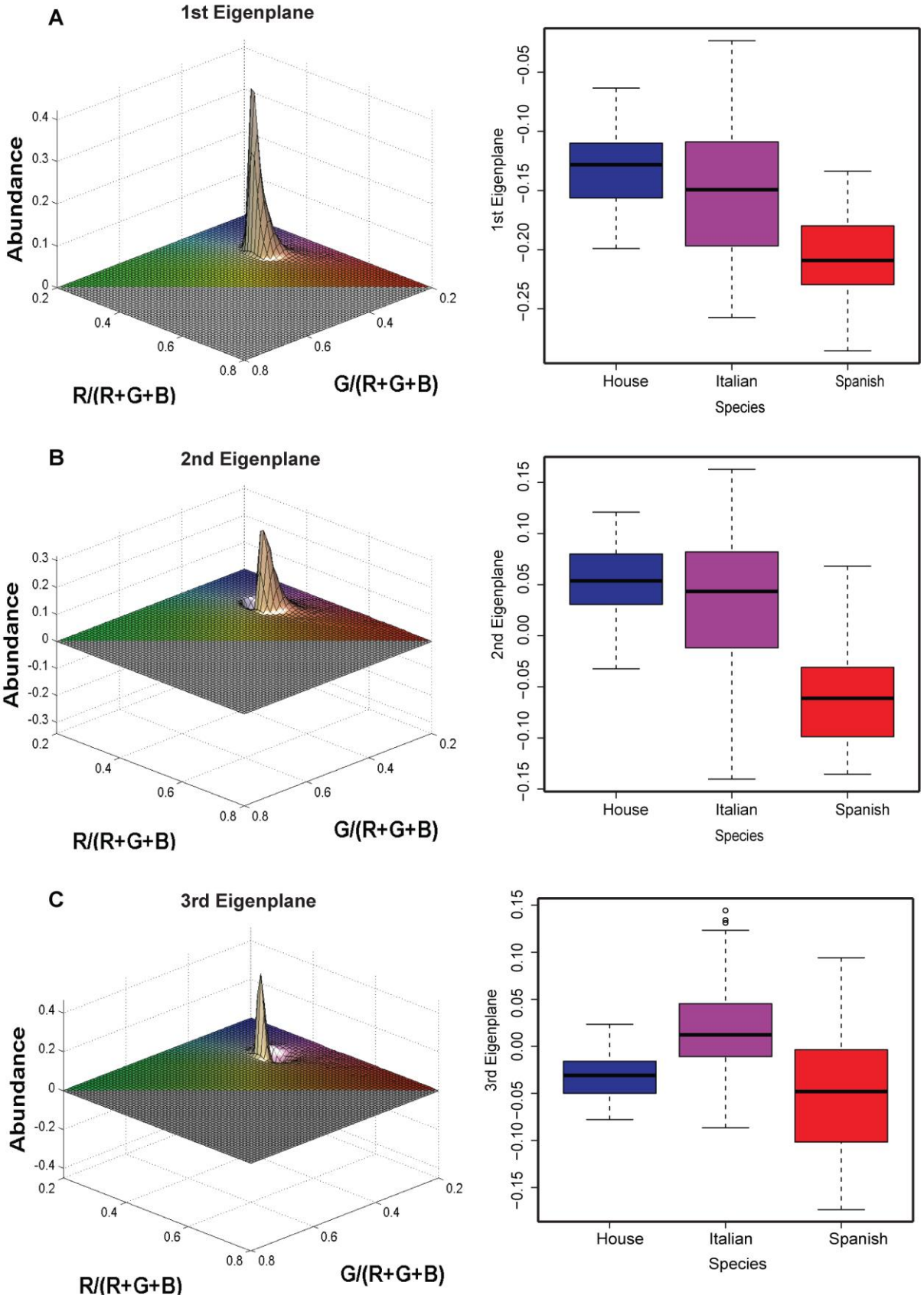

Figure S7. Back plumage chromaticity and the amount of variation between species for each eigenplane; shown in the 1st eigenplane (A), 2nd eigenplane (B), 3rd eigenplane (C) and 4th eigenplane (D).

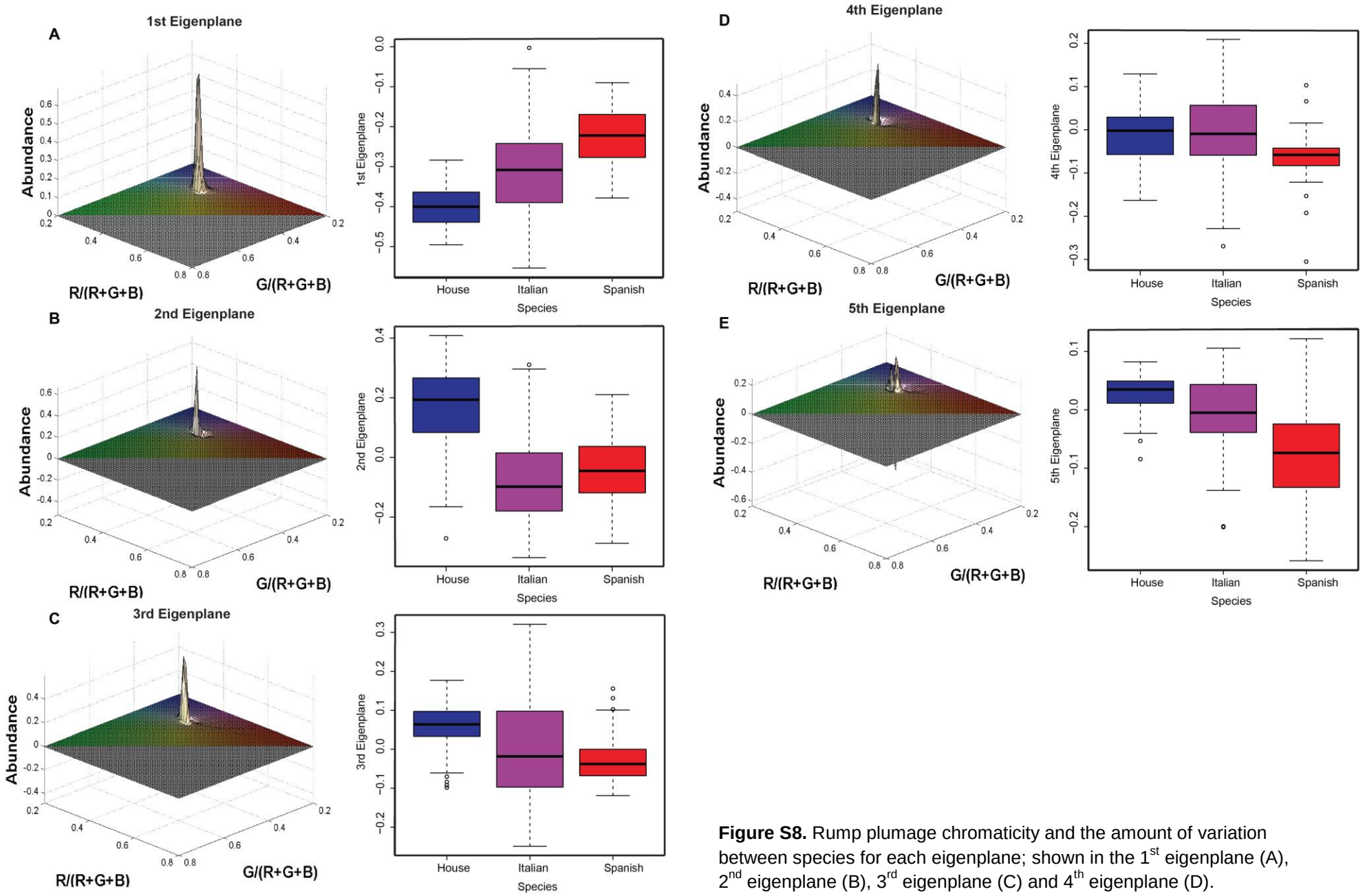

Figure S8. Rump plumage chromaticity and the amount of variation between species for each eigenplane; shown in the 1st eigenplane (A), 2nd eigenplane (B), 3rd eigenplane (C) and 4th eigenplane (D).

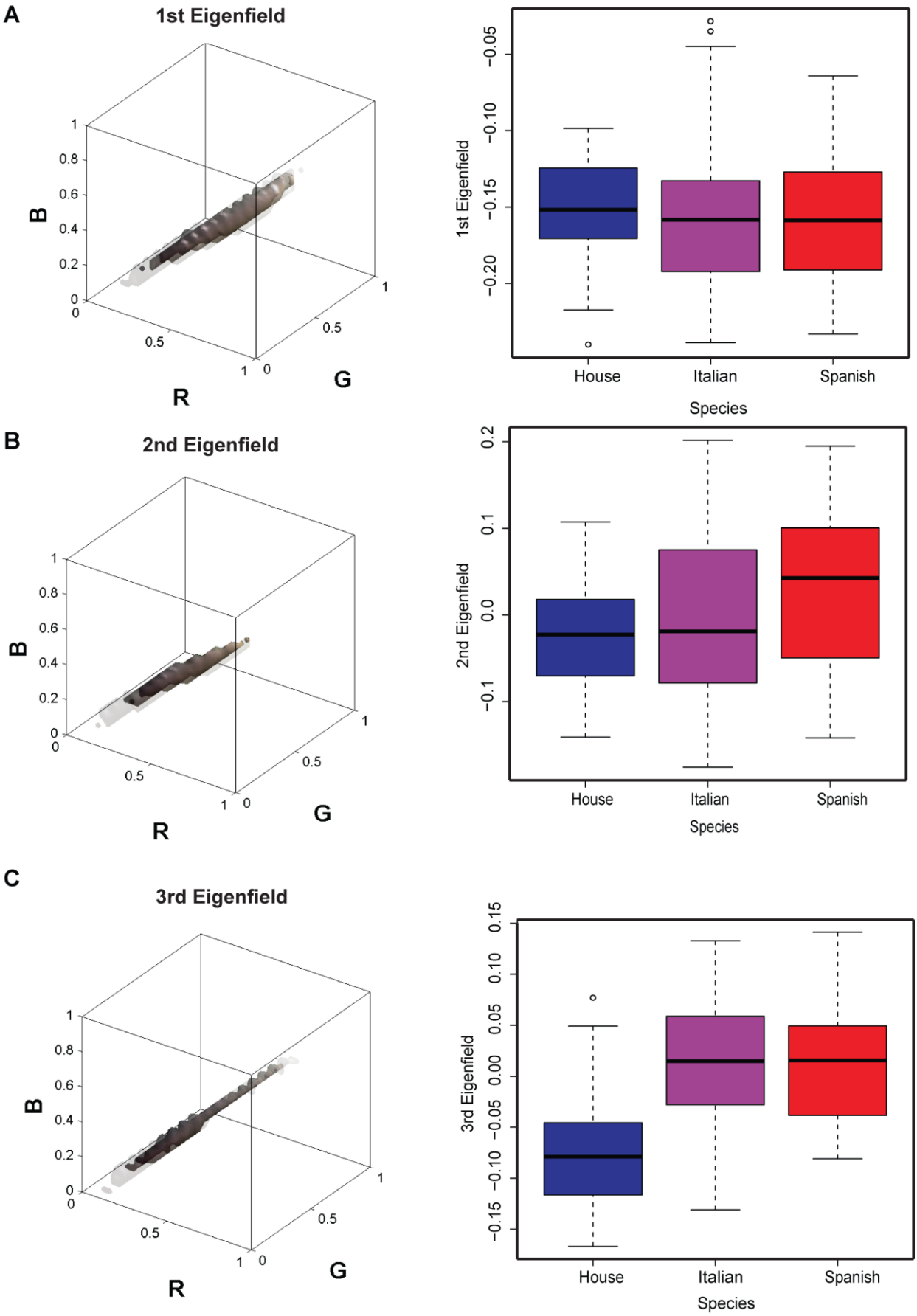

Figure S9. Cheek plumage chromaticity and the amount of variation between species for each eigenplane; shown in the 1st eigenplane (A), 2nd eigenplane (B), 3rd eigenplane (C) and 4th eigenplane (D).

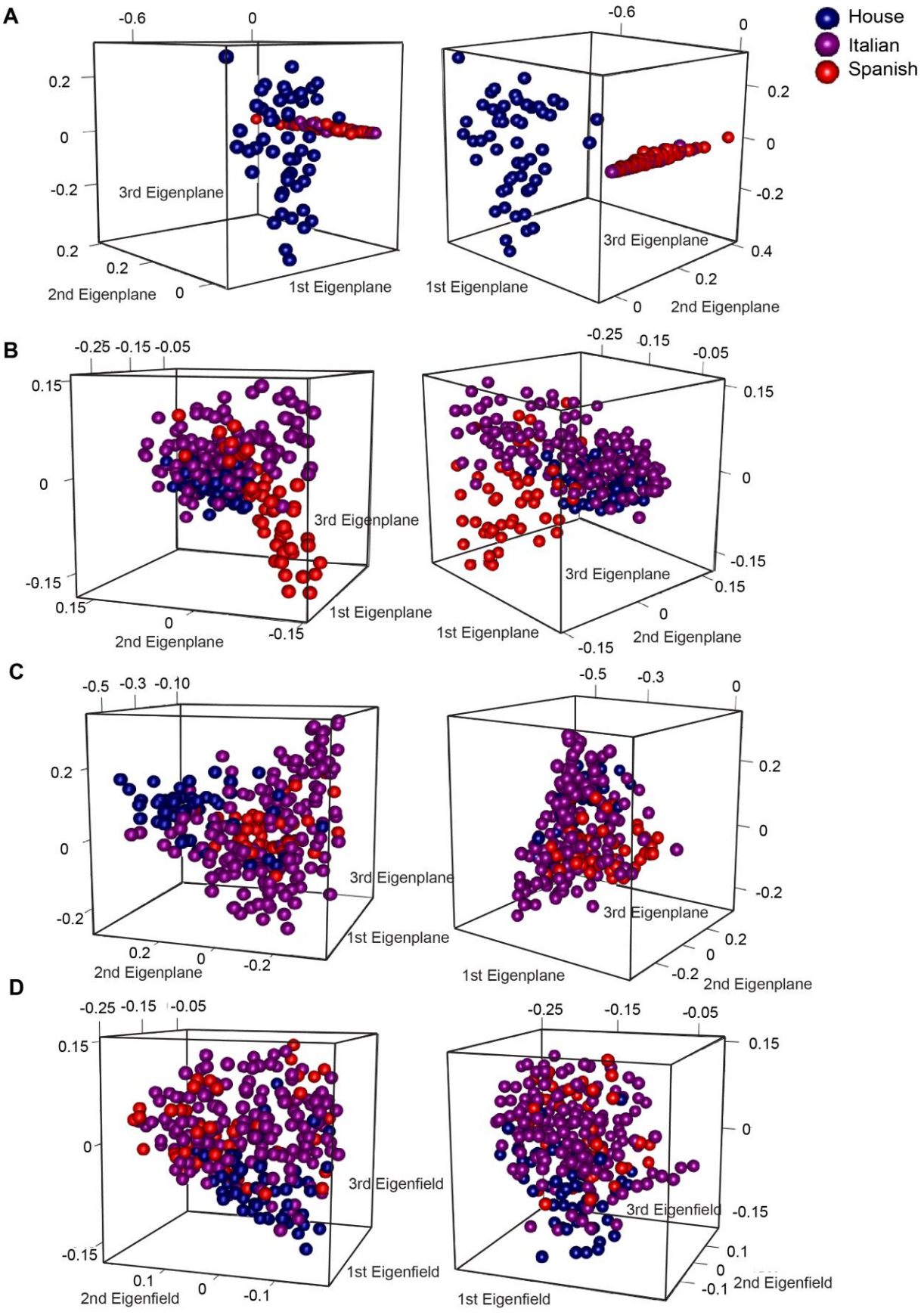

Figure S10. The amount of variation between sparrow species in the first three eigenplanes/fields per trait. Shown from two angles for crown (A), back (B), rump (C) and cheek plumage color (D).

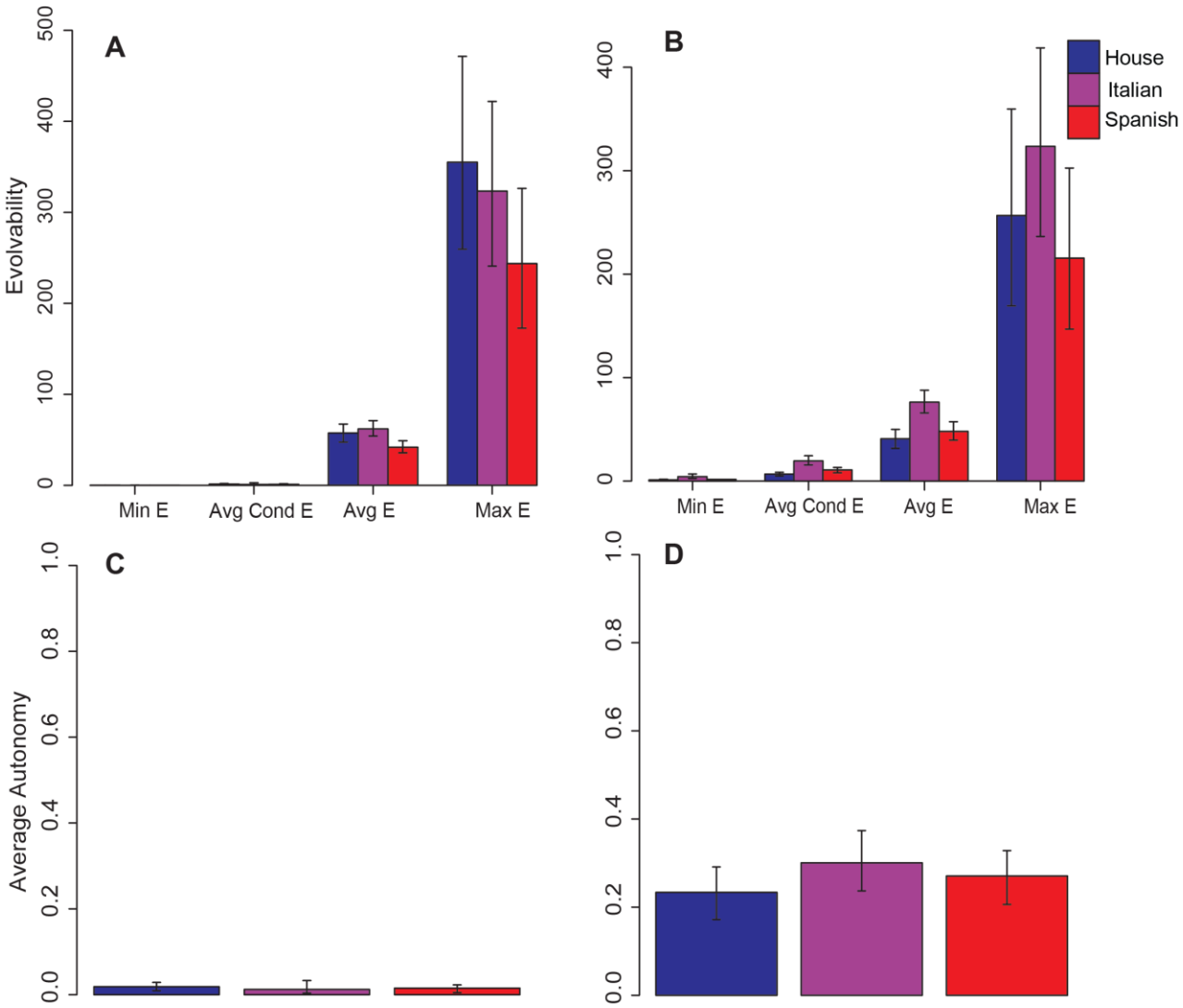

Figure S11. A comparison of evolvability statistics between all plumage traits and all plumage traits without crown. Crown plumage color is included in plots A and C, and excluded in plots B and D. Whiskers denote 2.5% and 9.75% quantiles.

Appendix II

Supplementary Tables

Table S1. The site and coordinates of each samples population for each of the respective species. Includes the number of photographed individuals, the number of individuals with its plumage color quantified for each of the respective traits, the number with all plumage traits quantified, and the number with at least one trait quantified from two photos. Coordinates are shown as latitude, longditude.

Species	Locality	Population	Coordinates	Photo	Crown	Back	Rump	Cheek	All Traits	Duplicate
P.italiae	Corsica	Muratello	41.58443, 9.19806	44	43	44	43	44	42	39
P.italiae		Pianiccia	42.14963, 9.50570	12	10	12	12	12	10	12
P.italiae		Tiuccia	42.07093, 8.73772	14	14	14	14	14	14	14
P.italiae	Crete	Mithinma Camping	35.50251, 23.70335	17	17	14	14	17	14	17
P.italiae		Istro	35.12661, 25.73300	18	18	18	18	18	18	18
P.italiae		Chania Hospital	35.47882, 24.01750	11	11	10	10	10	10	10
P.italiae	Sicily	Cos	37.02988, 14.52267	11	10	11	10	11	9	11
P.italiae		Enna	37.56516, 14.23891	18	18	18	18	18	18	18
P.italiae		Naxos	37.81052, 15.25778	26	26	26	26	26	26	26
P.domesticus	France	Tempetay	46.31286, 5.23190	12	12	12	12	12	12	10
P.domesticus		Sales	45.87932, 5.96063	26	26	26	26	26	26	18
P.domesticus	Switzerland	Lignieres	47.08322, 7.06720	15	15	15	15	15	15	13
P.hispaniolensis	Sardinia	Oniferi	40.27878, 9.16913	15	15	15	15	15	15	5
P.hispaniolensis	Kazakhstan	Kazakhstan	42.51754, 70.62668	14	14	14	14	14	14	7
P.hispaniolensis	Malta	Malta	35.88094, 14.44046	22	22	22	22	22	22	22
Total	-	-	-	275	271	271	269	274	265	240

Table S2. The amount of variation explained by each factor for all three species, all three island populations, house sparrows, Spanish sparrows and Italian sparrows of Corsica, Crete and Sicily. Between-species, between-island, between-population, between-individual variation and residuals are shown for each respective group. Residuals represent the amount of measurement error. Upper and lower quantiles are shown for the proportion between-individual variation, the proportion between-species variation and the proportion between-island variation.

Trait[1]	Group	Species	Island	Pop	Ind	Res	2.5%, 97.5% Proportion Ind	2.5%, 97.5% Proportion Species	2.5%, 97.5% Proportion Isl
All Traits	Species	767.30	-	58.20	200.73	96.89	0.18, 0.57	0.26, 0.78	-
	Italian	-	30.26	31.15	215.92	49.84	0.77, 0.93	-	0.02, 0.13
	House	-	-	29.28	148.91	220.45	0.88, 0.98	-	-
	Spanish	-	-	13.49	110.98	131.66	0.64, 0.97	-	-
	Corsica	-	-	6.54	220.76	110.54	0.93, 0.99	-	-
	Crete	-	-	61.70	217.55	88.99	0.45, 0.96	-	-
	Sicily	-	-	8.16	104.26	57.97	0.80, 0.98	-	-
Crown	Species	650.69	-	27.92	61.22	87.74	0.07, 0.35	0.49, 0.89	-
	Italian	-	17.77	22.29	54.44	55.70	0.35, 0.76	-	0.05, 0.53
	House	-	-	16.83	121.43	241.28	0.79, 0.99	-	-
	Spanish	-	-	65.15	22.76	56.57	0.05, 0.61	-	-
	Corsica	-	-	12.29	39.70	39.16	0.27, 0.89	-	-
	Crete	-	-	9.47	104.46	185.89	0.79, 0.97	-	-
	Sicily	-	-	17.61	22.74	50.36	0.21, 0.85	-	-
Back	Species	11.37	-	36.32	37.87	18.36	0.14, 0.43	0.11, 0.40	-
	Italian	-	11.58	45.75	45.19	15.89	0.18, 0.45	-	0.31, 0.71
	House	-	-	8.15	14.94	11.74	0.79, 0.99	-	-
	Spanish	-	-	29.64	38.92	32.68	0.14, 0.88	-	-
	Corsica	-	-	15.03	38.19	11.96	0.23, 0.91	-	-
	Crete	-	-	50.96	51.61	16.03	0.37, 0.95	-	-
	Sicily	-	-	5.62	47.66	25.37	0.73, 0.96	-	-
Rump	Species	209.62	-	134.97	103.99	207.18	0.26, 0.54	0.08, 0.79	-
	Italian	-	99.48	89.97	116.25	329.84	0.46, 0.85	-	0.04, 0.31
	House	-	-	26.28	211.06	166.73	0.58, 0.97	-	-
	Spanish	-	-	8.94	111.14	232.14	0.86, 0.98	-	-
	Corsica	-	-	32.08	141.26	314.12	0.46, 0.95	-	-
	Crete	-	-	32.08	135.57	437.52	0.16, 0.88	-	-
	Sicily	-	-	28.09	65.11	304.35	0.38, 0.91	-	-
Cheek	Species	19.44	-	184.64	461.07	308.47	0.26, 0.55	0.01, 0.20	-
	Italian	-	9.45	110.63	518.53	272.77	0.52, 0.90	-	0.17, 0.62
	House	-	-	5.76	282.04	386.21	0.94, 0.99	-	-
	Spanish	-	-	467.73	439.20	387.96	0.16, 0.88	-	-
	Corsica	-	-	13.09	619.22	332.55	0.90, 1.00	-	-
	Crete	-	-	5.55	543.37	255.19	0.46, 0.96	-	-
	Sicily	-	-	137.96	381.01	215.77	0.34, 0.97	-	-

1 'All Traits' includes only the first eigenplane/field from each trait.

Table S3. Median, upper and lower quantiles of evolvability statistics for all plumage traits (except crown) of each respective species and island population.

Measure	Species/Island	Median	2.5%, 9.75% Quantiles
Mean e	House	41.05	32.03, 50.26
	Italian	75.66	65.01, 86.78
	Spanish	44.25	36.71, 51.91
	Corsica	52.58	43.44, 64.59
	Crete	73.14	61.34, 85.35
	Sicily	47.87	40.09, 55.15
Mean c	House	6.21	4.38, 7.99
	Italian	17.65	13.85, 21.59
	Spanish	10.31	7.71, 12.81
	Corsica	10.22	7.50, 12.92
	Crete	14.40	9.39, 18.22
	Sicily	10.86	7.58, 14.02
Mean a	House	0.213	0.157, 0.266
	Italian	0.272	0.216, 0.330
	Spanish	0.266	0.199, 0.323
	Corsica	0.234	0.176, 0.294
	Crete	0.239	0.156, 0.305
	Sicily	0.257	0.191, 0.321
eBeta	House	19.30	10.44, 30.11
	Italian	42.89	27.04, 63.62
	Spanish	57.81	39.55, 76.14
	Corsica	45.46	29.82, 67.40
	Crete	39.40	24.61, 56.69
	Sicily	29.07	16.83, 44.32
cBeta	House	6.93	3.54, 11.79
	Italian	18.16	10.53, 27.53
	Spanish	19.42	8.98, 32.18
	Corsica	13.45	7.35, 20.49
	Crete	12.30	6.45, 18.75
	Sicily	8.46	4.68, 13.20

Mean e = mean evolvability
Mean c = mean conditional evolvability
Mean a = mean autonomy
eBeta = evolvability along the axis of parental differentiation
cBeta = conditional evolvability along the axis of parental differentiation

Table S4. Median, upper and lower quantiles of evolvability statistics for crown plumage color of each respective species and island population.

Measure	Species/Island	Median	2.5%, 9.75% Quantiles
Mean e	House	103.82	78.61, 142.31
	Italian	24.77	16.26, 37.76
	Spanish	27.11	20.63, 37.01
	Corsica	11.90	7.85, 18.16
	Crete	36.78	27.65, 51.03
	Sicily	14.44	10.33, 21.05
Mean c	House	2.67	1.78, 3.63
	Italian	1.35	0.82, 2.22
	Spanish	1.66	1.28, 2.15
	Corsica	0.79	0.59, 27.68
	Crete	2.26	1.73, 2.97
	Sicily	1.36	0.78, 1.86
Mean a	House	0.027	0.018, 0.038
	Italian	0.056	0.031, 0.103
	Spanish	0.064	0.046, 0.088
	Corsica	0.086	0.057, 0.130
	Crete	0.064	0.046, 0.089
	Sicily	0.107	0.059, 0.156
eBeta	House	132.15	90.47, 201.39
	Italian	5.63	3.27, 9.50
	Spanish	5.19	3.61, 7.83
	Corsica	4.03	2.41, 6.75
	Crete	7.00	4.70, 11.02
	Sicily	5.48	3.48, 8.95
cBeta	House	7.06	2.39, 10.69
	Italian	0.26	0.12, 0.68
	Spanish	0.34	0.24, 0.51
	Corsica	0.20	0.12, 0.33
	Crete	0.33	0.23, 0.52
	Sicily	0.50	0.16, 0.82

Mean e = mean evolvability
Mean c = mean conditional evolvability
Mean a = mean autonomy
eBeta = evolvability along the axis of parental differentiation
cBeta = conditional evolvability along the axis of parental differentiation

Table S5. Median, upper and lower quantiles of evolvability statistics for back plumage color of each respective species and island population.

Measure	Species/Island	Median	2.5%, 9.75% Quantiles
Mean e	House	9.58	7.41, 12.77
	Italian	33.75	24.14, 49.47
	Spanish	27.71	21.02, 38.36
	Corsica	18.44	12.65, 26.74
	Crete	22.53	17.24, 30.80
	Sicily	26.23	19.18, 37.76
Mean c	House	6.805	5.45, 8.59
	Italian	19.85	15.02, 26.73
	Spanish	13.51	10.80, 17.14
	Corsica	12.67	9.26, 17.02
	Crete	17.91	14.16, 23.08
	Sicily	15.10	11.72, 19.68
Mean a	House	0.705	0.581, 0.826
	Italian	0.632	0.487, 0.781
	Spanish	0.490	0.388, 0.604
	Corsica	0.717	0.570, 0.853
	Crete	8.935	0.671, 0.914
	Sicily	0.605	0.470, 0.742
eBeta	House	11.11	7.64, 16.85
	Italian	53.20	33.26, 85.89
	Spanish	9..26	6.45, 14.00
	Corsica	13.79	8.19, 23.74
	Crete	27.88	18.75, 44.09
	Sicily	46.46	29.92, 74.73
cBeta	House	5.31	3.67, 8.02
	Italian	25.98	17.06, 40.91
	Spanish	4.43	3.09, 6.61
	Corsica	8.64	5.37, 14.20
	Crete	19.82	13.42, 30.94
	Sicily	18.83	12.17, 30.39

Mean e = mean evolvability
Mean c = mean conditional evolvability
Mean a = mean autonomy
eBeta = evolvability along the axis of parental differentiation
cBeta = conditional evolvability along the axis of parental differentiation

Table S6. Median, upper and lower quantiles of evolvability statistics for rump plumage color of each respective species and island population.

Measure	Species/Island	Median	2.5%, 9.75% Quantiles
Mean e	House	68.51	25.02, 55.96
	Italian	117.60	47.63, 131.57
	Spanish	61.64	23.31, 50.75
	Corsica	81.59	46.58, 125.44
	Crete	119.9	83.27, 196.20
	Sicily	63.70	25.39, 64.80
Mean c	House	15.35	3.99, 8.86
	Italian	45.01	8.12, 27.37
	Spanish	27.45	7.89, 16.66
	Corsica	28.76	6.48, 20.53
	Crete	36.90	8.26, 18.65
	Sicily	21.11	2.83, 8.68
Mean a	House	0.268	0.096, 0.262
	Italian	0.376	0.086, 0.386
	Spanish	0.467	0.212, 0.506
	Corsica	0.363	0.071, 0.306
	Crete	0.310	0.055, 0.169
	Sicily	0.316	0.061, 0.243
eBeta	House	36.71	25.02, 55.96
	Italian	80.89	47.63, 131.57
	Spanish	33.48	23.31, 50.75
	Corsica	75.32	46.58, 125.44
	Crete	123.9	83.27, 196.20
	Sicily	39.84	25.39, 64.80
cBeta	House	5.90	3.99, 8.86
	Italian	14.86	8.12, 27.37
	Spanish	11.18	7.89, 16.66
	Corsica	12.10	6.48, 20.53
	Crete	12.08	8.26, 18.65
	Sicily	5.18	2.83, 8.68

Mean e = mean evolvability
Mean c = mean conditional evolvability
Mean a = mean autonomy
eBeta = evolvability along the axis of parental differentiation
cBeta = conditional evolvability along the axis of parental differentiation

Table S7. Median, upper and lower quantiles of evolvability statistics for cheek plumage color of each respective species and island population.

Measure	Species/Island	Median	2.5%, 9.75% Quantiles
Mean e	House	27.75	21.78, 36.58
	Italian	47.26	35.51, 64.97
	Spanish	46.28	35.76, 62.37
	Corsica	44.12	32.55, 62.45
	Crete	50.03	37.53, 70.26
	Sicily	40.46	29.85, 57.95
Mean c	House	21.55	17.17, 27.35
	Italian	34.07	26.00, 44.91
	Spanish	31.29	25.13, 39.52
	Corsica	30.48	22.81, 40.60
	Crete	29.98	23.57, 38.79
	Sicily	25.50	19.75, 33.42
Mean a	House	0.766	0.639, 0.881
	Italian	0.736	0.583, 0.873
	Spanish	0.683	0.563, 0.803
	Corsica	0.699	0.541, 0.842
	Crete	0.596	0.472, 0.732
	Sicily	0.650	0.512, 0.791
eBeta	House	21.84	14.97, 33.27
	Italian	27.42	16.37, 45.72
	Spanish	22.02	15.30, 33.28
	Corsica	28.29	17.26, 46.90
	Crete	24.36	16.38, 38.44
	Sicily	20.65	13.52, 33.19
cBeta	House	13.49	9.02, 20.41
	Italian	19.76	12.10, 32.82
	Spanish	15.97	11.17, 23.93
	Corsica	19.00	11.38, 32.05
	Crete	11.76	7.99, 18.40
	Sicily	13.84	9.03, 22.12

Mean e = mean evolvability
Mean c = mean conditional evolvability
Mean a = mean autonomy
eBeta = evolvability along the axis of parental differentiation
cBeta = conditional evolvability along the axis of parental differentiation

Table S8. Species and island differences in color variation in all traits from MANOVA models using significant SVD2D eigenplanes.

Group	Trait	Model[1]	Df[2]	V[3]	F[4]	numDf, denDF[5]	p-Value	AIC
Species	All Traits	Species	2, 262	1.532	50.70	32, 496	<2.2e-16	-14082
		IslandParent	6, 260	2.637	29.98	64, 992	<2.2e-16	-14831
		Population	14, 250	4.173	6.58	224, 3472	<2.2e-16	-15439
	Crown	Species	2, 267	0.922	56.71	8, 530	<2.2e-16	-4318
		IslandParent	4, 265	1.298	31.81	16, 1060	<2.2e-16	-4410
		Population	14, 255	1.769	14.44	56, 1020	<2.2e-16	-4555
		Bushy	1, 268	0.045	3.14	4, 265	0.01526	-3116
	Back	Species	2, 262	0.625	39.51	6, 522	<2.2e-16	-3600
		IslandParent	4, 260	1.068	35.95	12, 780	<2.2e-16	-3841
		Population	14, 250	1.428	16.23	42, 750	<2.2e-16	-3964
	Rump	Species	2, 262	0.703	28.06	10, 518	<2.2e-16	-4007
		IslandParent	4, 260	1.312	25.27	20, 1036	<2.2e-16	-4314
		Population	14, 250	1.754	9.65	70, 1250	<2.2e-16	-4471
	Cheek SVD2D	Species	2, 262	0.380	15.29	8,522	<2.2e-16	-2372
		IslandParent	4, 261	0.552	10.45	16, 1044	<2.2e-16	-2400
		Population	14, 251	1.031	6.22	56, 1004	<2.2e-16	-2604
	Cheek SVD3D	Species	2, 262	0.344	13.52	8, 520	<2.2e-16	-4309
		IslandParent	4,260	0.584	11.12	16, 1040	<2.2e-16	-4349
		Population	14, 250	1.005	5.99	56,1000	<2.2e-16	-4514
Islands	All traits	Island	2,158	1.313	17.19	32, 288	<2.2e-16	-9076
		Population	8,152	2.522	4.14	128, 1152	<2.2e-16	-9402
	Crown	Island	2,163	0.518	14.06	8, 322	<2.2e-16	-3149
		Population	8,157	0.848	5.28	32, 628	<2.2e-16	-3205
		Bushy	1,164	0.177	8.64	4, 161	2,433e-06	-2987
	Back	Island	2,158	0.584	21.57	6, 314	<2.2e-16	-2299
		Population	8,152	0,898	8,12	24, 456	<2,2e-16	-2346
	Rump	Island	2,158	0.743	18.34	10, 310	<2.2e-16	-2542
		Population	8,152	1.202	6.01	40, 760	<2.2e-16	-2641
	Cheek SVD2D	Island	2,158	0.234	5.21	8, 314	<2.2e-16	-1475
		Population	8,152	0.674	3.88	32, 612	<2.2e-16	-1573
	Cheek SVD3D	Island	2,158	0.407	9.97	8, 312	<2.2e-16	-2588
		Population	8,152	0.748	4.37	32, 608	<2.2e-16	-2665

1 IslandParent is a factor with Corsica, Crete, and Sicily, and all house and all Spanish sparrows.
2 Degrees of freedom for: fixed effect, residuals
3 Pillai-Bartlett Trace
4 Approximate F-value
5 The numerator degrees of freedom, the denominator degrees of freedom

Table S9. How similarly each species and island would respond to selection. Shown as a correlation matrix of the median random skewers correlation (r) between each combination of groups. 2.5% and 97.5% quantiles are displayed in parentheses next to the median r, representing resampling error.

Factor	Species	House	Italian
Species	Italian	0.411 (0.345, 0.455)	
	Spanish	0.284 (0.272, 0.298)	0.616 (0.567, 0.692)

Factor	Species/Isl	House	Corsica	Crete	Sicily
IslandParent	Corsica	0.457 (0.406, 0.519)			
	Crete	0.434 (0.395, 0.487)	0.845 (0.803, 0.868)		
	Sicily	0.319 (0.269, 0.358)	0.599 (0.531, 0.669)	0.675 (0.639, 0.717)	
	Spanish	0.276 (0.243, 0.310)	0.658 (0.616, 0.712)	0.655 (0.613, 0.681)	0.611 (0.562, 0.655)

Table S10. Significance of similarity of species' and islands' response to selection. Shown as a correlation matrix of the median probability (p) between each combination of groups. 2.5% and 97.5% quantiles are displayed in parentheses next to the median p, representing resampling error.

Factor	Species	House	Italian
Species	Italian	0.844 (0.661, 0.991)	
	Spanish	1.000 (0.998, 1.000)	0.008 (0.000, 0.061)

Factor	Species/Isl	House	Corsica	Crete	Sicily
IslandParent	Corsica	0.650 (0.260, 0.900)			
	Crete	0.800 (0.460, 0.930)	0.000 (0.000, 0.000)		
	Sicily	0.996 (0.980, 1.000)	0.016 (0.000, 0.190)	0.000 (0.000, 0.000)	
	Spanish	1.000 (1.000, 1.000)	0.000 (0.000, 0.010)	0.000 (0.000, 0.010)	0.010 (0.000, 0.080)